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Research paper

Synthesis of novel quinoline–based 4,5–dihydro–1*H*–pyrazoles as potential anticancer, antifungal, antibacterial and antiprotozoal agents

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ABSTRACT

A new series of *N*–substituted 2–pyrazolines **9a**–**f**, **10a**–**f**, **11a**–**f**, **12a**–**f** and **13a**–**f** were obtained from the cyclocondensation reaction of [(7–chloroquinolin–4–yl)amino]chalcones **8a**–**f** with hydrazine hydrate and its derivatives. Fourteen of the synthesized compounds including the starting chalcones were selected by US National Cancer Institute (NCI) for testing their anticancer activity against 60 different human cancer cell lines, with the most important GI_{50} values ranging from 0.28 to 11.7 μM (0.13–6.05 μg/mL) and LC_{50} values ranging from 2.6 to > 100 μM (1.2 to > 51.7 μg/mL), for chalcones **8a**,**d** and pyrazolines **10c**,**d**. All compounds were assessed for antibacterial activity against wild type and multidrug resistant gram negative and gram positive bacteria, with MIC values ranging from 31.25 to 500 μg/mL. Additionally, the novel compounds were tested for antifungal and antiparasitic properties. Although these compounds showed mild activity against *Candida albicans*, chalcones **8a** and **8e** showed high activity against *Cryptococcus neoformans* with $MIC_{50} = 7.8$ μg/mL. For anti–*Plasmodium falciparum* activity the 2–pyrazoline **11b** was the most active with $EC_{50} = 5.54$ μg/mL. Regarding the activity against *Trypanosoma cruzi*, compound **10a** was highly active with $EC_{50} = 0.70$ μg/mL. Chalcone **8a** had good activity against *Leishmania panamensis* amastigotes with $EC_{50} = 0.79$ μg/mL.

1. Introduction

The pharmacological properties of the quinoline ring are well illustrated by the large number of commercially available drugs containing this heterocyclic system [1]. These compounds have been studied for decades and structure–activity relationship analyses have been performed to determine their biological effects [2]. Most studies on 4–aminoquinolines showed that the 7–chloro–4–aminoquinoline ring present in pharmacologically active substances displays a broad range of biological activities [3–6] and is determinant for antimalarial activity, because it accumulates into the digestive vacuole of the *Plasmodium* parasite and inhibits β–hematin formation by accumulation of the FPIX–4–aminoquinoline complex, a highly toxic moiety which induces parasite death [7–14]. Therefore, including this pharmacophore in the design of novel heterocyclic compounds might improve their biological activity.

Chalcones have shown broad spectrum of biological activities as antifungal [15], antiparasitic [16–19], anticancer [20] and antioxidant [21] compounds. They have also shown immunomodulatory [22], anti–invasive [23,24] and anti–inflammatory effects [25–28]. However their importance can also be associated with their recognized utility as intermediate compounds in the synthesis of pyrazolines [29], which have also been largely studied for their pharmacological activities. Pyrazolines (also called dihydropyrazoles) are known by their activities including anticancer [30],

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anti–inflammatory [31], antiparasitic [32], antidepressive, anticonvulsant [33], antimicrobial [34] and antinociceptive effects [35]. They have also been recognized as nitric oxide synthase inhibitors used in Alzheimer's and Huntington's diseases and inflammatory arthritis [36].

Regarding the synthesis and biological activities of 2–pyrazolines [37–39], in this study we are reporting the efficient synthesis of five series of novel quinoline–based 4,5–dihydro–1*H*–pyrazoles. The synthesized compounds including their synthetic intermediates quinoline–based chalcones were tested against a panel of cancer cell lines in the Developmental Therapeutics Program (DTP) at the National Cancer Institute (NCI). Given the emergence of antimicrobial and antiparasitic resistance [40], all synthesized compounds were also tested for *in vitro* antifungal, antibacterial and antiprotozoal activities.

2. Results and discussion

2.1. Chemistry

By using a previously reported methodology [41], 2–(3–nitrophenyl)–1,3–dioxolane **3** was synthesized and subjected to reduction in methanol with hydrazine monohydrate. Then, the precursor 3–(7–chloroquinolin–4–ylamino)benzaldehyde **6** was obtained in 98% yield through a selective nucleophilic aromatic substitution (S_NAr) of the 4–chlorine atom on the 4,7–dichloroquinoline **5** with amine **4** previously synthetized. Then a Claisen–Schmidt condensation reaction between aldehyde **6** and substituted acetophenones **7a**–**f** led the novel quinoline–based chalcones **8a**–**f** (Scheme 1), in good yields (71–90%).

The structure elucidation of compounds **8a**–**f** was performed from analysis of their spectroscopic data (FTIR, ^{1}H NMR, ^{13}C NMR and mass spectrometry). The IR spectrum of compound **8a** used as representative compound of this series, showed an absorption band at 1659 cm^{-1} associated to the stretching vibration of the carbonyl group from the α,β–unsaturated fragment. In the ^{1}H NMR spectrum of compound **8a**, two doublets at 7.78 and 7.95 ppm with coupling constant ^3J = 15.6 Hz assigned to H_β and H_α respectively are observed, indicating the *E* configuration for the carbon–carbon double bond.

Treatment of chalcones **8a**–**f** with hydrazine monohydrate in ethanol under reflux for 10 min, and further treatment with acetic anhydride or formic acid, at room temperature for 10 min (Scheme 2), afforded the acetylated and formylated products **9a**–**f** and **10a**–**f** respectively, in good yields (75–90%).

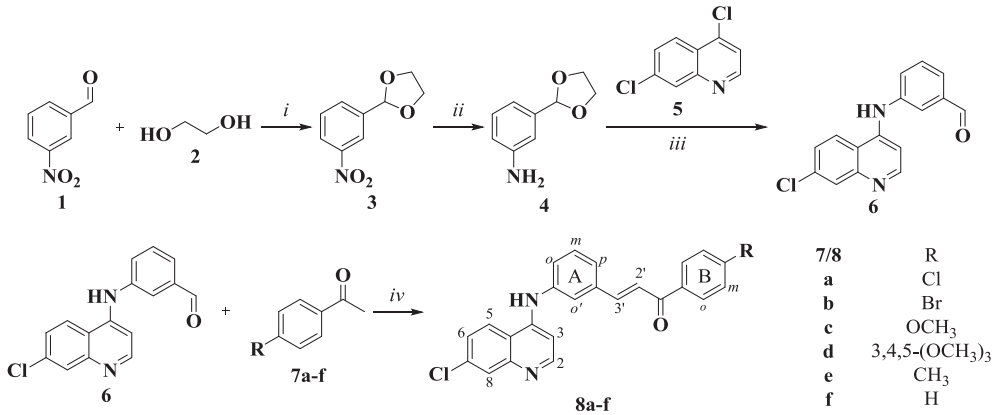
The IR spectrum of compound **9a** as a representative of the series **9a**–**f** and **10a**–**f**, showed an absorption band at 3352 cm^{-1} associated to the stretching vibration of the NH bond. Regarding the ^{1}H NMR spectrum of compound **9a**, it showed a singlet at 2.32 ppm, assigned to the methyl of the acetyl–inserted group. Instead, ^{1}H NMR spectrum of compound **10a** showed a singlet at 8.93 ppm associated to the proton of the formyl–inserted group. In all cases, the two methylene protons of $C_{4'}$ and the stereogenic proton in $C_{5'}$ generated an AMX spin system. For compound **9a**, the signal associated to proton H_A appears as a double–doublet at 3.24 ppm with coupling constant values $^2J_{AM}$ = 18.2 Hz and $^3J_{AX}$ = 4.9 Hz; for proton H_M the double–doublet appears at 3.92 ppm, with coupling constant values $^2J_{AM}$ = 18.2 Hz and $^3J_{MX}$ = 12.0 Hz while the corresponding signal of the H_X proton appeared as a double–doublet at 5.63 ppm with coupling constant values $^3J_{MX}$ = 12.0 Hz and $^3J_{AX}$ = 4.9 Hz. Furthermore, the ^{13}C NMR spectrum of compound **9a** showed all the expected signals.

Reaction of the synthesized chalcones **8a**–**f** with phenylhydrazine and its derivatives in methanol under reflux led to the *N*–aryl pyrazolines **11a**–**f**, **12a**–**f** and **13a**–**f** (Scheme 3) in acceptable to excellent yields. The new pyrazolines were fully characterized by spectroscopic techniques such as FTIR and 1D and 2D–NMR (see Experimental section).

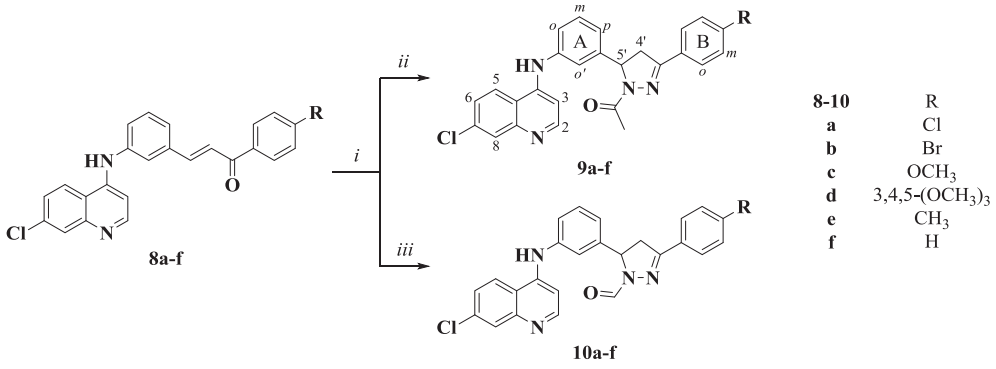
The IR spectrum of compound **12a** as a representative of series **11a**–**f**, **12a**–**f** and **13a**–**f**, showed an absorption band at 3345 cm^{-1} associated to the stretching vibration of the NH bond. Regarding the ^{1}H NMR spectrum, it showed two doublets at 7.03 and 7.24 ppm, each one integrating for 2H assigned to the protons of the 4–Cl–aryl–inserted group. The signal associated to proton H_A appears as a double–doublet at 3.25 ppm with coupling constant values $^2J_{AM}$ = 17.7 Hz and $^3J_{AX}$ = 6.1 Hz; for proton H_M the double–doublet appears at 3.99 ppm, with coupling constant values $^2J_{AM}$ = 17.7 Hz and $^3J_{MX}$ = 12.3 Hz while the corresponding signal of the H_X proton appeared as a double–doublet at 5.64 ppm with coupling constant values $^3J_{MX}$ = 12.3 Hz and $^3J_{AX}$ = 6.1 Hz. Furthermore, the ^{13}C NMR spectrum of compound **12a** showed all the expected signals.

2.2. Anticancer activity

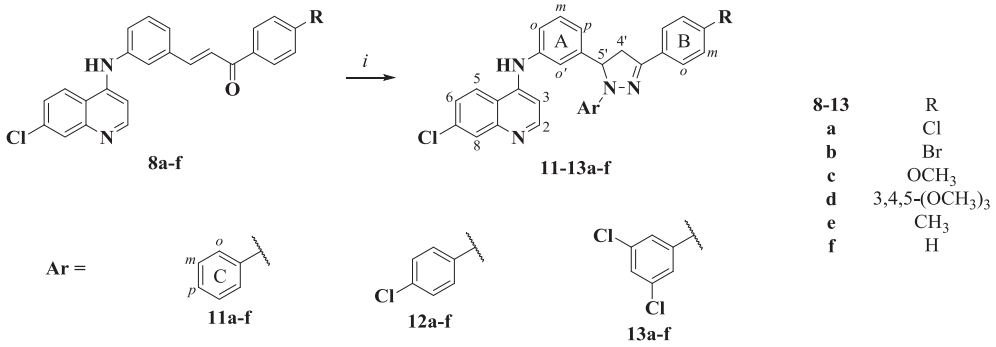
For a more comprehensive analysis of the results, we grouped all compounds into two series: (A) that includes chalcones **8a**–**f** and (B) that comprise pyrazolines **9a**–**f**, **10a**–**f**, **11a**–**f**, **12a**–**f** and **13a**–**f**. Series (B) was in turn divided into two groups: (B_1) that comprises compounds with an acetyl (**9a**–**f**) or a formyl substituent (**10a**–**f**) on N–1 of the 2–pyrazoline ring; and (B_2) that includes compounds with an unsubstituted (**11a**–**f**) or substituted phenyl group



Scheme 1. General methodology for the synthesis of aldehyde **6** and chalcones **8a**–**f**: *i* = PTSA, toluene, reflux, 24 h *ii* = $NH_2NH_2{\cdot}H_2O$, Ni–Raney, MeOH, reflux, 1 h *iii* = 1) EtOH, reflux, 3 h. 2) HCl conc. *iv* = KOH, MeOH, rt, 12 h.



Scheme 2. General methodology for the synthesis of compounds **9a**–**f** and **10a**–**f**: *i* = $NH_2NH_2 \cdot H_2O$, EtOH, reflux, 10 min *ii* = Ac_2O (for **9a**–**f**), rt, 10 min *iii* = HCOOH (for **10a**–**f**), rt, 10 min.



Scheme 3. Synthesis of compounds **11a**–**f**, **12a**–**f** and **13a**–**f**: *i* = $C_6H_5NHNH_2$ (for **11a**–**f**), 4–$ClC_6H_4NHNH_2 \cdot HCl$ (for **12a**–**f**), 3,5–*di*Cl–$C_6H_3NHNH_2 \cdot HCl$ (for **13a**–**f**), MeOH, reflux, 3 h.

[4–Cl (**12a**–**f**) or 3,5–*di*Cl (**13a**–**f**)], on N–1 of the 2–pyrazoline ring.

As a preliminary screening, structures of all new compounds (i.e. **6**, **8a**–**f**, **9a**–**f**, **10a**–**f**, **11a**–**f**, **12a**–**f** and **13a**–**f**) were submitted to the Developmental Therapeutics Program (DTP) at National Cancer Institute (NCI) for evaluation of their anticancer activity against different human cell lines. Fourteen (i.e. **8a**,**c**,**d**; **9a**,**c**; **10a**,**c**,**d**; **11a**,**c**; **12a**,**c**,**d** and **13a**) compounds were selected and subjected to the preliminary anticancer evaluation against the 60 cancer cell lines at a single dose of 10 μM after 48 h of incubation. The output from the single dose screening was analyzed by the COMPARE program (data not shown). It was observed that only the chalcones and pyrazolines with Cl and OCH_3 on the ring B were selected for anticancer evaluation suggesting that the substituents on this ring are modulators of the anticancer activity.

The results of this first screening showed that compounds **8c**,**d** and **10a**,**c** passed to a second evaluation step in order to determine their cytostatic activity against 58 cancer cell lines from lung, colon, brain, breast, ovary, kidney, prostate and leukemia and melanoma cell panels. None of the compounds of series B_2 (**11a**,**c**; **12a**,**c**,**d** and **13a**) were selected for further testing suggesting that the substitution with a phenyl group on N–1 of the 2–pyrazoline ring decreases the anticancer activity of the compounds. It is interesting to note that in a recent paper [37], we found that analogues of compounds **11a**–**f** and **12a**–**f**, with scheleton type B_2 but with *N*–phenyl substituent attached to the vicinal N, exhibit anticancer activity suggesting that the position of ring C is important for anticancer activity.

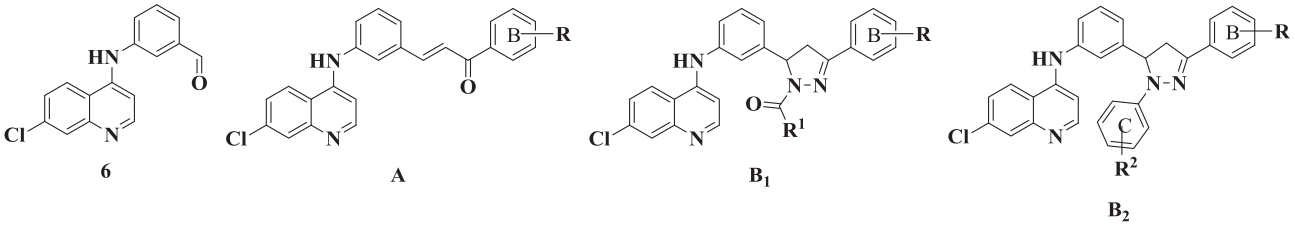
The results were expressed as follows according to previously published protocols: GI_{50}, which is the molar concentration of the compounds required to inhibit 50% of the growth of cell lines (relative to untreated cells), and LC_{50}, which is a parameter of cytotoxicity that reflects the molar concentration needed to kill 50% of the cells [42]. The active compounds were evaluated at several concentrations (100, 10, 1.0, 0.1, and 0.01 μM) for a 48–h protocol in which sulforhodamide B (SRB) protein assay was used to estimate cell growth according to protocols described elsewhere [43,44]. As shown in Table 1, compounds **8c**,**d** and **10a**,**c** showed great values of GI_{50} against several cell lines, some of them lower than 1.00 μM. Compound **8c** showed GI_{50} values from 0.49 to 3.85 μM (0.20–1.60 μg/mL, 6 of them < 1.00 μM) and LC_{50} values from 23.7 to > 100 μM (9.8 to > 41.5 μg/mL). The best cytostatic activity of **8c** was displayed against the SR leukemia cell line with $GI_{50} = 0.49$ μM (0.20 μg/mL) and the best cytotoxic activity was shown against the U251 CNS cancer cell line with $LC_{50} = 23.7$ μM (9.8 μg/mL), better than adriamycin, the standard drug. Compound **8d** was the most active with GI_{50} values from 0.31 to 2.49 μM (0.15–1.18 μg/mL) (20 of them < 1.00 μM) and LC_{50} values from 2.6 to > 100 μM (1.2 to > 47.5 μg/mL). The best cytostatic activity of **8d** was displayed against the RPMI–8226 leukemia cell line with $GI_{50} = 0.31$ μM (0.15 μg/mL) and the best cytotoxic activity was shown against the SK–OV–3 ovarian cancer cell line with $LC_{50} = 2.6$ μM (1.2 μg/mL), better than adriamycin. Compound **10a** showed GI_{50} values from 0.28 to 9.42 μM (0.13–4.35 μg/mL) (12 of them < 1.00 μM) and LC_{50} values from 5.1 to > 100 μM (2.3 to > 46.1 μg/mL). The best cytostatic activity of **10a** was displayed against the MDA–MB–468 breast cancer cell line with $GI_{50} = 0.28$ μM (0.13 μg/mL) and the best cytotoxic activity was shown against the SK–MEL–5 melanoma cell line with $LC_{50} = 5.1$ μM (2.3 μg/mL). Compound **10c** showed GI_{50} values from 0.37 to 11.7 μM (0.19–6.05 μg/mL) (5 of them < 1.00 μM) and LC_{50} values from 17.4 to > 100 μM (9.0 to > 51.7 μg/mL). The best activity was shown against the

Table 1
In vitro cytotoxic activities of compounds **8c**,**d** and **10a**,**c** expressed as growth inhibition and lethal concentration of cancer cell lines and compared with the standard drug adriamycin.[a]

Panel cell line	Compounds								Doxorubicin (adriamycin), NSC 123127	
	8c		8d		10a		10c		100 μM[d]	
	GI_{50}[b]	LC_{50}[c]	GI_{50}	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}
Leukemia										
CCRF−CEM	2.88	>100	0.36	>100	1.42	>100	2.14	>100	0.08	100.00
HL−60(TB)	2.98	>100	1.82	>100	1.27	>100	2.03	33.6	0.12	89.33
K−562	1.64	83.8	0.40	>100	0.39	>100	0.51	>100	0.19	100.00
MOLT−4	2.90	>100	1.79	>100	1.13	>100	2.37	>100	0.03	100.00
RPMI−8226	0.57	>100	0.31	>100	0.61	>100	1.36	>100	0.08	100.00
SR	0.49	>100	0.32	>100	0.40	>100	0.58	>100	0.03	100.00
Non−small cell lung cancer										
A549/ATCC	2.78	>100	1.48	11.5	2.03	>100	4.63	>100	0.06	100.00
EKVX	1.75	>100	1.62	–	1.59	>100	2.81	>100	0.41	47.97
HOP−62	1.44	>100	0.68	6.06	1.21	70.0	2.11	67.7	0.07	67.61
HOP−92	1.09	>100	1.47	>100	1.45	>100	3.41	>100	0.10	42.27
NCI−H226	1.78	>100	1.27	6.85	1.87	>100	3.13	>100	0.05	6.40
NCI−H23	1.49	>100	1.67	8.10	1.07	>100	1.87	>100	0.15	13.15
NCI−H322M	2.69	>100	1.52	7.08	5.52	>100	6.51	>100	0.54	67.76
NCI−H460	2.23	>100	1.91	>100	1.86	>100	2.73	>100	0.02	51.29
NCI−H522	1.88	>100	0.59	6.23	1.12	66.7	1.33	61.9	0.03	2.80
Colon cancer										
COLO 205	3.85	>100	1.34	>100	1.43	21.4	2.40	60.6	0.18	4.33
HCC−2998	–	>100	1.72	5.99	3.12	>100	11.7	>100	0.26	21.68
HCT−116	0.84	>100	0.35	3.98	1.20	17.2	1.84	24.3	0.08	54.58
HCT−15	2.04	>100	0.52	–	1.36	>100	2.13	>100	6.46	100.00
HT29	1.64	>100	0.32	5.51	1.28	56.4	1.65	67.4	0.12	67.45
KM12	2.45	>100	0.44	–	1.22	>100	2.18	>100	0.27	92.68
SW−620	1.98	>100	0.45	>100	1.61	>100	1.73	>100	0.09	58.61
CNS cancer										
SF−268	2.47	>100	1.24	–	2.73	>100	4.79	>100	0.10	30.48
SF−295	0.69	>100	1.42	6.47	0.50	>100	1.24	>100	0.10	69.98
SF−539	1.70	>100	1.36	5.27	1.69	35.1	2.34	39.7	0.12	27.23
SNB−19	2.26	>100	1.18	5.08	4.19	>100	5.11	>100	0.04	49.77
SNB−75	1.51	>100	1.31	>100	1.70	>100	2.56	>100	0.07	3.30
U251	1.73	23.7	0.91	5.19	2.47	53.9	2.77	51.1	0.04	30.62
Melanoma										
LOX IMVI	1.58	>100	0.37	3.95	1.29	9.05	2.52	39.0	0.07	50.35
MALME−3M	1.79	>100	1.46	7.80	1.27	76.6	2.04	>100	0.12	3.97
M14	1.60	>100	1.41	–	1.55	>100	2.34	67.5	0.18	4.05
MDA−MB−435	2.13	>100	0.47	–	0.38	>100	0.37	>100	0.25	9.57
SK−MEL−28	1.87	>100	1.51	5.98	3.15	>100	8.12	67.1	0.21	15.92
SK−MEL−5	1.32	>100	1.38	5.18	0.54	5.05	0.92	17.4	0.08	0.49
UACC−257	2.07	>100	1.43	6.53	1.47	>100	3.06	>100	0.14	8.15
UACC−62	1.19	>100	1.03	5.40	1.14	87.8	2.41	68.1	0.14	1.31
Ovarian cancer										
IGROV1	1.44	>100	1.32	>100	1.44	>100	3.71	>100	0.17	100.00
OVCAR−3	1.69	>100	0.39	3.85	1.10	83.3	1.97	63.7	0.39	84.33
OVCAR−4	1.00	–	1.26	5.26	0.64	79.8	1.54	>100	0.37	74.30
OVCAR−5	2.60	>100	1.70	6.37	9.42	>100	11.3	>100	0.41	100.00
OVCAR−8	2.26	>100	0.52	7.07	1.92	>100	3.29	>100	0.10	43.25
NCI/ADR−RES	1.16	>100	0.41	>100	0.78	>100	1.66	>100	7.16	100.00
SK−OV−3	2.22	>100	1.94	2.61	2.90	>100	3.33	>100	0.22	100.00
Renal cancer										
786−0	1.48	77.7	0.56	4.89	2.96	62.7	3.43	79.8	0.13	51.64
A498	3.00	>100	1.54	>100	0.98	>100	1.35	>100	0.10	1.90
ACHN	1.73	>100	1.24	4.99	2.64	>100	4.59	>100	0.08	100.00
RXF 393	1.82	>100	1.08	5.67	1.40	>100	1.66	59.2	0.10	4.69
SN12C	1.30	>100	1.16	–	3.99	>100	4.45	>100	0.07	72.44
TK−10	1.64	>100	1.45	6.11	2.34	>100	4.24	61.7	0.38	89.95
UO−31	1.03	–	0.57	4.42	1.70	>100	3.25	>100	0.49	26.18
Prostate cancer										
PC−3	2.51	>100	1.48	>100	1.69	>100	2.87	>100	0.32	87.10
DU−145	2.45	>100	1.46	–	2.80	>100	3.62	>100	0.11	100.00
Breast cancer										
MCF7	1.82	>100	0.61	5.24	0.87	>100	1.59	>100	0.04	64.12
MDA−MB−231/ATCC	1.91	>100	1.81	–	2.74	>100	4.00	>100	0.51	34.75
HS 578T	3.18	>100	2.49	>100	3.23	>100	3.92	>100	0.33	85.70
BT−549	1.87	>100	1.23	6.59	1.78	>100	1.93	>100	0.23	21.33
T−47D	0.66	>100	1.22	8.26	0.82	>100	1.39	>100	0.06	85.70
MDA−MB−468	0.58	>100	1.13	–	0.28	>100	0.42	63.8	0.05	2.52

[a] Data obtained from NCI's *in vitro* disease−oriented human cancer cell lines screen in μM.
[b] GI_{50} was the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. Determined at five concentration levels (100, 10, 1.0, 0.1, and 0.01 μM).
[c] LC_{50} is a parameter of cytotoxicity that reflects the molar concentration needed to kill 50% of the cells.
[d] The values of activity against human cancer cell lines displayed by adriamycin correspond to the reported by NCI at highest concentration of 100 μM. Please visit: https://dtp.cancer.gov/dtpstandard/cancerscreeningdata/index.jsp. The most active compounds were highlighted in grey.

Table 2
Antifungal activity expressed as MIC_{80} and MIC_{50} obtained for compounds **6**, **8a–f**, **9a–f**, **10a–f**, **11a–f**, **12a–f** and **13a–f** against *C. albicans* and *C. neoformans*.



			R	R^1	R^2	*C. albicans*		*C. neoformans*	
						MIC_{80}	MIC_{50}	MIC_{80}	MIC_{50}
		6	–	–	–	≥250	250	250	250
A		**8a**	4–Cl	–	–	≥250	62.5	7.8	7.8
		8b	4–Br	–	–	≥250	250	250	125
		8c	4–OCH_3	–	–	≥250	250	≥250	≥250
		8d	3,4,5–$(OCH_3)_3$	–	–	≥250	≥250	≥250	≥250
		8e	4–CH_3	–	–	≥250	125	15.62	7.8
		8f	–	–	–	≥250	≥250	62.5	31.25
B	B_1	**9a**	4–Cl	CH_3	–	≥250	≥250	250	125
		9b	4–Br	CH_3	–	≥250	≥250	≥250	250
		9c	4–OCH_3	CH_3	–	≥250	≥250	≥250	250
		9d	3,4,5–$(OCH_3)_3$	CH_3	–	≥250	250	≥250	250
		9e	4–CH_3	CH_3	–	≥250	250	250	125
		9f	–	CH_3	–	≥250	250	≥250	250
		10a	4–Cl	H	–	≥250	250	≥250	31.25
		10b	4–Br	H	–	≥250	250	250	62.5
		10c	4–OCH_3	H	–	≥250	≥250	≥250	125
		10d	3,4,5–$(OCH_3)_3$	H	–	≥250	≥250	≥250	≥250
		10e	4–CH_3	H	–	≥250	≥250	≥250	125
		10f	–	H	–	≥250	≥250	≥250	250
	B_2	**11a**	4–Cl	–	H	≥250	≥250	≥250	≥250
		11b	4–Br	–	H	≥250	≥250	≥250	≥250
		11c	4–OCH_3	–	H	≥250	≥250	≥250	≥250
		11d	3,4,5–$(OCH_3)_3$	–	H	≥250	250	≥250	250
		11e	4–CH_3	–	H	≥250	≥250	≥250	≥250
		11f	–	–	H	≥250	250	≥250	250
		12a	4–Cl	–	4–Cl	≥250	≥250	≥250	250
		12b	4–Br	–	4–Cl	≥250	≥250	≥250	≥250
		12c	4–OCH_3	–	4–Cl	≥250	≥250	≥250	250
		12d	3,4,5–$(OCH_3)_3$	–	4–Cl	≥250	≥250	≥250	≥250
		12e	4–CH_3	–	4–Cl	≥250	≥250	≥250	≥250
		12f	–	–	4–Cl	≥250	≥250	≥250	250
		13a	4–Cl	–	3,5–*di*Cl	≥250	≥250	≥250	≥250
		13b	4–Br	–	3,5–*di*Cl	≥250	≥250	≥250	≥250
		13c	4–OCH_3	–	3,5–*di*Cl	≥250	≥250	≥250	≥250
		13d	3,4,5–$(OCH_3)_3$	–	3,5–*di*Cl	≥250	≥250	≥250	≥250
		13e	4–CH_3	–	3,5–*di*Cl	≥250	≥250	≥250	≥250
		13f	–	–	3,5–*di*Cl	≥250	≥250	≥250	≥250

MDA–MB–435 melanoma cell line with GI_{50} = 0.37 μM (0.19 μg/mL) and against the SK–MEL–5 melanoma cell line with LC_{50} = 17.4 μM (9.0 μg/mL). These findings make compounds **8c**,**d** and **10a**,**c**, promising targets to perform structural modifications and improve their anticancer activities.

2.3. Antifungal activity

Considering that some nitrogen–bearing heterocyclic compounds containing fragments like 2–pyrazoline moiety have demonstrated antifungal activity in previous reports [37,45–48], compounds **6**, **8a–f**, **9a–f**, **10a–f**, **11a–f**, **12a–f** and **13a–f** were tested for antifungal activity against two clinically important fungal species, *C. albicans* and *C. neoformans*. *C. neoformans* is an opportunistic fungus and causes significant mortality and morbidity in HIV–infected patients [49]. Moreover, *C. albicans* is the fourth leading cause of nosocomial bloodstream infection (BSI) in intensive care units, causing fatal invasive candidiasis in a high percentage of patients [50].

To assess antifungal activities, the broth microdilution method M27–A3 for yeasts of the Clinical and Laboratory Standards Institute was used [51]. Growth inhibition was determined by testing all compounds at the concentration range 3.9–250 μg/mL (Table 2 and Supplementary Table 1).

Table 2 shows the minimum concentration that inhibits 80% or 50% of fungal growth (MIC_{80} and MIC_{50} respectively) of *C. albicans* and *C. neoformans*. Most of the clinically used antifungals are active at a MIC level ≤10 μg/mL [52]. Almost all compounds of series B (**9a–f**, **10a–f**, **11a–f**, **12a–f** and **13a–f**) lack of antifungal activity suggesting that cyclization of the double bond of the chalcone derivatives **8a–f** into an azole ring (**9a–f**, **10a–f**, **11a–f**, **12a–f** and **13a–f**) abrogates the antifungal effects of the compounds. In a recent paper [37] we found that analogues of compounds **11a–f** and **12a–f**, with scheleton type B_2 but with *N*–phenyl substituent

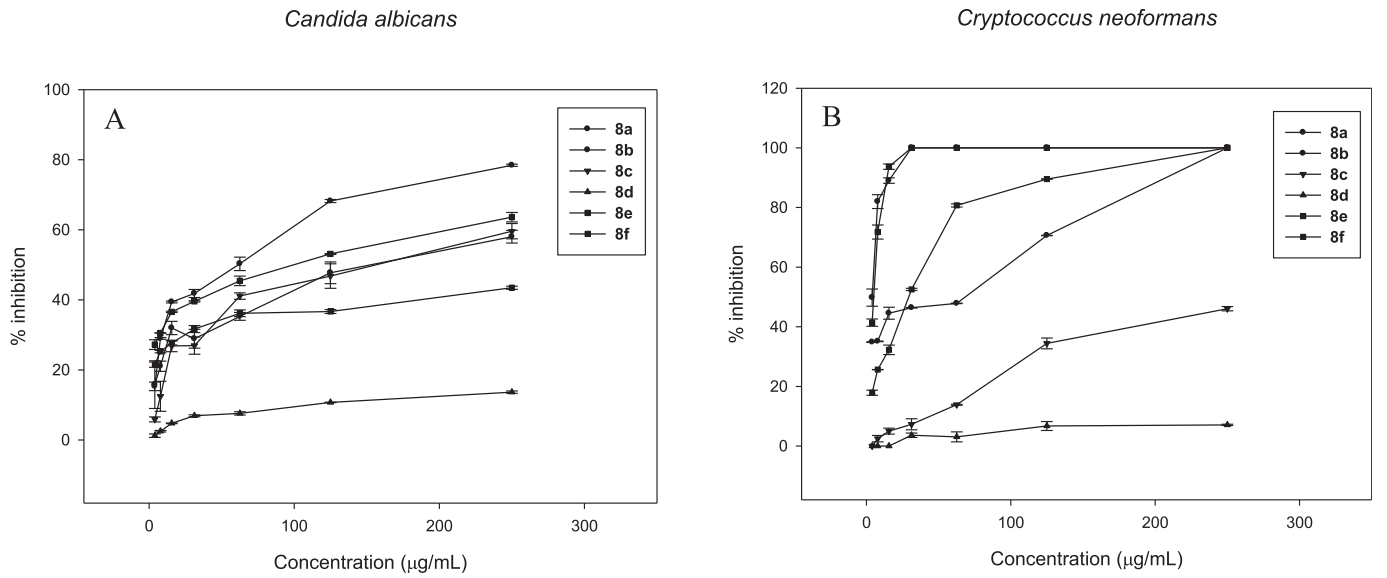


Fig. 1. Comparative dose–response curves of compounds **8a**–**f** against (A) *C. albicans* ATCC 10231 and (B) *C. neoformans* ATCC 32264. Amphotericin B, used as the control, displayed 100% of inhibition at all concentrations tested (curve not shown).

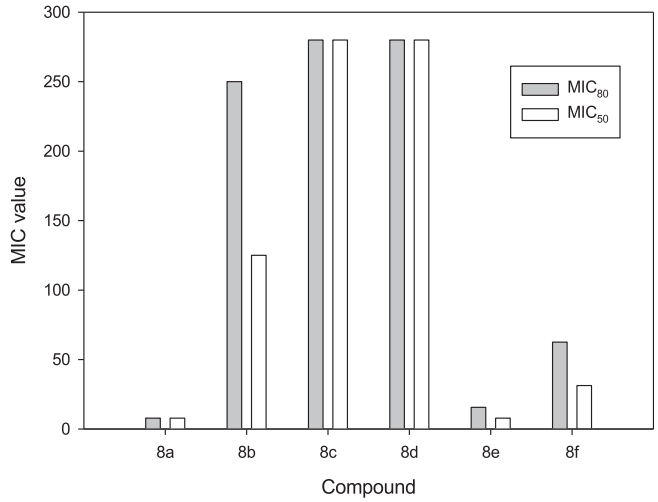


Fig. 2. Comparative MIC_{80} and MIC_{50} values (μg/mL) for each compound of the series **8a**–**f** against *C. neoformans* ATCC 32264.

attached to the vicinal N, displayed relevant antifungal activity suggesting that the position of the ring C is important for antifungal activity too. In addition, the results obtained with the aldehyde **6** clearly show that the 7–chloro–4–aminoquinoline nucleus does not confer antifungal activity.

For the A series, the best activity was displayed by both, the compounds **8a** (4–Cl benzene) and **8e** (4–CH_3 benzene) against *C. neoformans* (MIC_{50} = 7.8 μg/mL). Instead, the unsubstituted **8f** was moderately active (MIC_{50} = 31.25 μg/mL); **8b** (4–Br benzene) was marginally active (MIC_{50} = 125 μg/mL) and **8c** (4–OCH_3 benzene) and **8d** [3,4,5– $(OCH_3)_3$ benzene] were inactive (MIC_{50} > 250 μg/mL) against *C. neoformans* and marginally active (MIC ≥ 250 μg/mL) against *C. albicans*. Fig. 1 shows the percentages of inhibition for all compounds of series **8**, at the different concentrations tested, against *C. albicans* (Fig. 1A) and *C. neoformans* (Fig. 1B).

As shown in Fig. 1, *C. neoformans* was more sensitive than *C. albicans* when tested with compounds **8a**–**f**, being **8a** and **8e** the most active compounds with low MIC_{80} (7.8 and 15.6 μg/mL respectively) and MIC_{50} (7.8 μg/mL for both compounds). Fig. 2 compares the MIC_{80} and MIC_{50} values for each compound of the series **8a**–**f** against *C. neoformans*.

In Fig. 2, it is shown that **8a** and **8e** possess the lower MICs against *C. neoformans*, followed by **8f**. These findings clearly highlight that the quinoline–based chalcones **8a** and **8e** could be used as leads for developing new series with promising anticryptococcal activity for further development.

2.4. Antibacterial activity

All the novel compounds were screened for their *in vitro* antibacterial activity against gram–positive and gram–negative bacteria including wild type and emerging multidrug resistant strains. The minimum inhibitory and bactericidal concentrations (MIC and MBC) were determined and reported in Table 3. Compound **6** (the parent benzaldehyde chloroquinoline) was the most active, showing inhibitory activity against *Staphylococcus aureus* ATCC 25923 (methicillin sensitive, MIC 62.5 μg/mL), vancomycin–intermediate *Staphylococcus aureus* (VISA) (MIC 125 μg/mL) and *Escherichia coli* ATCC 25922 (MIC 62.5 μg/mL). This compound also showed discrete inhibition against a carbapenemase–positive *Klebsiella pneumoniae* BAA 1705 (MIC 250 μg/mL) which represents one of the therapeutically challenging multidrug resistant strains and an emerging worldwide threat. Compound **9d**, a pyrazoline bearing a trimethoxyphenyl moeity, selectively inhibited *Neisseria gonorrhoeae* growth with MIC of 31.25 μg/mL. None of the compounds seemed to be active against *P. aeruginosa* or *K. pneumoniae* ATCC 700603. The results obtained with the aldehyde **6** show that the 7–chloro–4–aminoquinoline nucleus confer antibacterial activity, but this activity is abolished when the nucleus is functionalized with benzene rings B and C. Compound **9d** is an exception to this observation probably by the presence of the trimethoxy substituent. Although the MICs for the active compounds are much higher than those for licensed antibiotics, they may be a starting point to develop compounds with improved antibacterial activity particularly against resistant strains.

Table 3
In vitro antibacterial activity (MIC/MBC values in μg/mL).

	S. aureus ATCC 25923	*S. aureus* ATCC 43300	*VISA*	*E. coli* ATCC 25922	*P. aeruginosa*	*K. pneumoniae* ATCC 700603	*K. pneumoniae* BAA 1705	*N. gonorrhoeae* ATCC 31426
6	62.5/500	500/>1000	125/500	62.5/500	>1000	>1000	250/>1000	500
8a–f	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
9a–c	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
9d	>1000	>1000	>1000	>1000	>1000	>1000	>1000	31.25
9e–f	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
10a–f	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
11a–f	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
12a–f	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
13a–f	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000

Active compounds were highlighted in grey.

2.5. Cytotoxic activity in human U–937 macrophages

All compounds were tested first for toxicity on U–937 human cells at four serial dilution concentrations (200, 50, 12.5 and 3.125 μg/mL) to determine their 50% Lethal Concentration (LC_{50}) (Table 4). All compounds of series A and B_1 (**6**, **8a–f**, **9a–f** and **10a–f**) and few compounds of series B_2 (**11b**, **11c**, **11e** and **12b**) exhibiting LC_{50} < 100 μg/mL were considered as potentially cytotoxic whereas almost all compounds of the series B_2 (**11a**, **11d**, **11f**, **12a**, **12c–f** and **13a–f**) showed a LC_{50} > 200 μg/mL and therefore classified as potentially non cytotoxic. Amphotericin B showed a LC_{50} = 36.0 μg/mL (potentially cytotoxic) and benznidazole had a LC_{50} = 180.0 μg/mL (mildly cytotoxic). It is clear from Table 4 that the substitution with a phenyl group on N–1 of the 2–pyrazoline ring decreases the cytotoxic activity of the compounds and the decrease is greater when this ring is attached to one or two chlorine atoms. The results obtained with the aldehyde **6** show that the 7–chloro–4–aminoquinoline nucleus confer cytotoxic activity, but this activity is diminished when the nucleus is functionalized with rings A and B and specially with ring C. However, these results cannot be misinterpreted, since amphotericin B has high cytotoxicity and is still a commercial drug. Therefore the results should be confirmed with *in vivo* studies.

2.6. Antiplasmodial activity

All compounds were screened *in vitro* for antiplasmodial activity on asynchronic cultures of *P. falciparum* (3D7 strain, sensitive to chloroquine) at four serial diluted concentration (100, 25, 6.25 and 1.56 μg/mL) to determine the Effective Concentration 50 (EC_{50}) (See Table 5).

Most compounds, except **8a**, **9a**, **9b**, **12c**, **12d** and **13e**, were highly active against *P. falciparum* ($EC_{50} \leq 25$ μg/mL). Compound **11b** was the most active (EC_{50} = 5.54 μg/mL). The EC_{50} obtained with the aldehyde **6** and **CQ** show that the 7–chloro–4–aminoquinoline nucleus confer antiplasmodial activity, and also that this activity can be improved when the nucleus is functionalized with ring A in the 4–amino group. Although almost all active compounds showed lower EC_{50} than **CQ**, these values do not vary significantly respect to compound **6** suggesting that the antiplasmodial activity is given by 7–chloro–4–aminoquinoline nucleus and the ring A and it is not improved with the functionalization with rings B and C. Most compounds of series B_2 (**11a**, **11d**, **11f**, **12a**, **12e**, **12f**, **13a–d** and **13f**) showed better EC_{50} than **CQ**, and also LC_{50} > 200 μg/mL, these results suggest that those compounds have potential as antimalarial drug, therefore could be further studied *in vivo* for drug development in malaria therapy.

2.7. Antitrypanosomal activity

Antitrypanosomal activity was screened against intracellular amastigotes of *Trypanosoma cruzi* (Tulahuen strain) at four serial dilutions. The activity of these compounds (in terms of EC_{50}) is summarized in Table 6. The EC_{50} ranged from 0.70 to 22.58 μg/mL for active compounds ($EC_{50} \leq 25$ μg/mL). Compound **10a** was highly active against *T. cruzi* (EC_{50} = 0.70 μg/mL). It is clear from Table 6,

Table 4
In vitro cytotoxic activity of novel synthetic compounds **6**, **8a–f**, **9a–f**, **10a–f**, **11a–f**, **12a–f** and **13a–f**.

Comp.	LC_{50}[a]	Comp.	LC_{50}	Comp.	LC_{50}
6	0.6 ± 0.1	**10a**	0.6 ± 0.1	**12a**	>200
8a	0.7 ± 0.1	**10b**	0.6 ± 0.1	**12b**	35.7 ± 2.9
8b	0.7 ± 0.1	**10c**	0.3 ± 0.1	**12c**	>200
8c	8.1 ± 1.3	**10d**	17.4 ± 1.7	**12d**	>200
8d	0.9 ± 0.2	**10e**	1.3 ± 0.2	**12e**	>200
8e	2.0 ± 0.4	**10f**	1.3 ± 0.2	**12f**	>200
8f	1.5 ± 0.3	**11a**	>200	**13a**	>200
9a	17.0 ± 1.9	**11b**	9.2 ± 0.6	**13b**	>200
9b	10.2 ± 1.1	**11c**	28.0 ± 1.7	**13c**	>200
9c	4.9 ± 0.4	**11d**	>200	**13d**	>200
9d	5.1 ± 0.8	**11e**	1.4 ± 0.1	**13e**	>200
9e	4.4 ± 0.2	**11f**	>200	**13f**	>200
9f	5.8 ± 0.9	**Amph**[b]	36.0 ± 7.5	**Benz**[c]	180 ± 3.7

Data represent the mean value ± standard deviation in μg/mL. [a] LC_{50}: Lethal concentration 50% of promonocitic human cell U–937. [b] Amph: Amphotericin B (antileishmanial drug control). [c] Benz: Benznidazole (antitrypanosomal drug control).

Table 5
In vitro antimalarial screening of novel synthetic compounds **6**, **8a–f**, **9a–f**, **10a–f**, **11a–f**, **12a–f** and **13a–f**.

Comp.	EC_{50}[a]	Comp.	EC_{50}	Comp.	EC_{50}
6	14.16 ± 1.58	**10a**	24.74 ± 3.59	**12a**	10.26 ± 0.00
8a	208.59 ± 34.77	**10b**	15.68 ± 1.54	**12b**	10.26 ± 0.00
8b	10.32 ± 0.09	**10c**	16.13 ± 2.23	**12c**	44.56 ± 2.10
8c	10.54 ± 0.46	**10d**	24.28 ± 2.22	**12d**	44.56 ± 2.10
8d	14.12 ± 1.22	**10e**	16.20 ± 1.24	**12e**	13.69 ± 0.26
8e	10.26 ± 0.00	**10f**	17.67 ± 0.51	**12f**	10.26 ± 0.00
8f	10.26 ± 0.00	**11a**	11.71 ± 0.80	**13a**	11.35 ± 0.13
9a	26.33 ± 0.99	**11b**	5.54 ± 0.32	**13b**	14.40 ± 1.21
9b	50.08 ± 2.30	**11c**	13.66 ± 0.29	**13c**	10.39 ± 0.15
9c	13.82 ± 1.15	**11d**	15.50 ± 0.63	**13d**	10.89 ± 0.95
9d	22.95 ± 1.48	**11e**	13.53 ± 0.37	**13e**	65.17 ± 13.03
9e	17.51 ± 1.28	**11f**	13.36 ± 0.33	**13f**	12.55 ± 0.42
9f	12.66 ± 0.37	**CQ**[b]	18.9 ± 1.7		

Data represent the mean value ± standard deviation in μg/mL. [a] EC_{50}: Effective Concentration 50: The exact amount of compounds that decreases 50% of intracellular parasite. [b] CQ: Chloroquine diphosphate salt (antimalarial control).

Table 6
In vitro testing of antitrypanosomal activity for novel compounds **6**, **8a**–**f**, **9a**–**f**, **10a**–**f**, **11a**–**f**, **12a**–**f** and **13a**–**f**.

Comp.	EC_{50}[a]	Comp.	EC_{50}	Comp.	EC_{50}
6	1.15 ± 0.19	**10a**	0.70 ± 0.04	**12a**	55.75 ± 5.38
8a	0.72 ± 0.13	**10b**	1.24 ± 0.23	**12b**	29.47 ± 4.43
8b	1.35 ± 0.19	**10c**	>0.15	**12c**	44.56 ± 2.10
8c	3.29 ± 0.38	**10d**	7.22 ± 0.84	**12d**	22.58 ± 1.96
8d	1.09 ± 0.04	**10e**	0.96 ± 0.04	**12e**	21.92 ± 0.83
8e	>1	**10f**	>0.6	**12f**	15.11 ± 2.24
8f	>0.8	**11a**	16.21 ± 3.08	**13a**	19.08 ± 2.54
9a	6.57 ± 0.43	**11b**	6.67 ± 0.34	**13b**	28.27 ± 3.17
9b	3.70 ± 0.26	**11c**	86.82 ± 27.91	**13c**	31.05 ± 6.50
9c	3.44 ± 0.12	**11d**	21.59 ± 3.81	**13d**	21.94 ± 2.63
9d	4.08 ± 0.78	**11e**	1.09 ± 0.01	**13e**	49.24 ± 1.63
9e	1.62 ± 0.15	**11f**	39.20 ± 5.22	**13f**	32.65 ± 1.27
9f	4.26 ± 0.43	**Benz**[b]	10.7 ± 2.0		

Data represent the mean value ± standard deviation in μg/mL. [a] EC_{50}: Effective Concentration 50: The exact amount of compounds that decreases 50% of intracellular *T. cruzi* amastigotes. [b] Benz: Benznidazole (antitrypanosomal drug control).

that almost all compounds of series A and B_1 (**8a**–**f**, **9a**–**f** and **10a**–**f**) are highly active against *T. cruzi*, but the activity is diminished when the N–1 of the 2–pyrazoline ring is substituted with a phenyl group. The EC_{50} obtained with the aldehyde **6** show that the 7–chloro–4–aminoquinoline nucleus confer antitrypanosomal activity but this activity is diminished with the functionalization with rings A, B and C. The results suggest that compounds **11a** (EC_{50} = 16.21 μg/mL), **11d** (EC_{50} = 21.59 μg/mL), **12d** (EC_{50} = 22.58 μg/mL), **12e** (EC_{50} = 21.92 μg/mL), **12f** (EC_{50} = 15.11 μg/mL), **13a** (EC_{50} = 19.08 μg/mL) and **13d** (EC_{50} = 21.94 μg/mL) with LC_{50} > 200 μg/mL have potential as antitrypanosomal drugs and therefore their potential as leading compounds should be further studied.

2.8. Antileishmanial activity

Compounds were screened for antileishmanial activity in intracellular amastigotes of *Leishmania* (*V*) *panamensis* (MHOM/CO/87/UA140–EpiR–GFP strain). As done previously with antiplasmodial and antitrypanosomal activities, the EC_{50} was determined after exposure to four concentrations (Table 7). Most compounds evidenced good activity against *Leishmania* amastigotes with EC_{50} varying from 0.79 to 22.67 μg/mL for active compounds ($EC_{50} \leq 25$ μg/mL). The best antileishmanial activity was displayed by compound **8a** which is active at 0.79 μg/mL. Most compounds of series A and B_1 (**8a**–**f**, **9a**–**f** and **10b**) are highly active against *T. cruzi*, but the activity is diminished when the N–1 of the 2–pyrazoline ring is substituted with a phenyl group. The results obtained with the aldehyde **6** show that the 7–chloro–4–aminoquinoline nucleus confer antileishmanial activity but this activity is diminished with the functionalization with rings A, B and C. None of the compounds had EC_{50} lower than amphotericin B, however compounds **11a**, **11d**, **11f**, **12d**, **12f** and **13a**–**f** were active and showed LC_{50} > 200 μg/mL (better than amphotericin B) suggesting that those compounds have potential as antileishmanial drug reducing the side effects of amphotericin B and therefore could be considered as a hit for *in vivo* studies, especially compound **13b** (EC_{50} = 2.77 μg/mL).

Table 7
In vitro antileishmanial activity of synthetic compounds **6**, **8a**–**f**, **9a**–**f**, **10a**–**f**, **11a**–**f**, **12a**–**f** and **13a**–**f**.

Comp.	EC_{50}[a]	Comp.	EC_{50}	Comp.	EC_{50}
6	0.96 ± 0.18	**10a**	280.92 ± 86.57	**12a**	91.39 ± 11.28
8a	0.79 ± 0.09	**10b**	2.41 ± 0.14	**12b**	15.99 ± 0.03
8b	0.88 ± 0.11	**10c**	33.14 ± 66.29	**12c**	27.73 ± 0.77
8c	2.65 ± 0.37	**10d**	6.79 ± 0.39	**12d**	21.26 ± 3.20
8d	5.92 ± 0.66	**10e**	2.19 ± 0.10	**12e**	49.40 ± 9.11
8e	1.20 ± 0.11	**10f**	1.72 ± 0.16	**12f**	15.67 ± 2.49
8f	2.54 ± 0.45	**11a**	11.69 ± 0.79	**13a**	12.05 ± 0.41
9a	5.53 ± 0.14	**11b**	3.59 ± 0.09	**13b**	2.77 ± 0.50
9b	3.66 ± 0.16	**11c**	6.93 ± 0.27	**13c**	12.61 ± 0.70
9c	2.98 ± 0.19	**11d**	13.41 ± 0.53	**13d**	16.49 ± 1.30
9d	1.96 ± 0.14	**11e**	1.83 ± 0.19	**13e**	18.12 ± 2.25
9e	1.55 ± 0.14	**11f**	22.67 ± 0.53	**13f**	17.84 ± 2.05
9f	3.67 ± 0.10	**Amph**[b]	0.05 ± 0.01		

Data represent the mean value ± standard deviation in μg/mL.
[a] EC50: Effective Concentration 50: The exact amount of compound that decreases 50% of intracellular parasite growth. b Amph: Amphotericin B (antileishmanial drug control).

3. Conclusions

A new series of novel 4,5–dihydro–1*H*–pyrazoles **9a**–**f**, **10a**–**f**, **11a**–**f**, **12a**–**f** and **13a**–**f** were efficiently synthetized in good yields by the reaction of hydrazine and its derivatives with quinoline–based α,β–unsaturated carbonyl compounds **8a**–**f**. Anticancer activity studies showed that four compounds (**8c**,**d** and **10a**,**c**) showed prominent values of GI_{50} against several cell lines, some of them lower than 1.00 μM. The studies on antifungal activity showed that compounds **8a** and **8e** completely inhibited *C. neoformans* growth at 31.25 μg/mL. We also found compound **9d** active against *N. gonorrhoeae* (MIC 31.25 μg/mL) a bacteria becoming a resistant superbug and a major public health problem. The parent compound **6** showed inhibition of MSSA, VISA and *E. coli*. For antimalarial activity 2–pyrazoline **11b** was the most active with EC_{50} = 5.54 μg/mL. Regarding to antitrypanosomal activity, the EC_{50} ranged from 0.70 to 22.58 μg/mL for active compounds, while compound **10a** was highly active with EC_{50} = 0.70 μg/mL. Antileishmanial activity studies revealed that some compounds were active with EC_{50} varying from 0.79 to 22.67 μg/mL for active compounds. The best antileishmanial activity was displayed by compound **8a** which is active at 0.79 μg/mL. In short, in this paper are reported several novel compounds exhibiting relevant *in vitro* anticancer and antiprotozoal activity that worth be further studied *in vivo*.

4. Experimental

4.1. General

Reagents and solvents used were obtained from commercial sources. Melting points were measured using a Stuart SMP10 melting point device and are uncorrected. FTIR spectrum were obtained with a Shimadzu IRAffinity–1. The ^{1}H and ^{13}C NMR spectrum were run on a BRUKER DPX 400 spectrometer operating at 400 and 100 MHz respectively, using DMSO–d_6 as solvent and TMS as internal standard. The mass spectrum was obtained on a SHIMADZU–GCMS–QP2010 spectrometer operating at 70 eV. The elemental analyses were obtained using a Thermo Finnigan Flash EA1112 CHN (STIUJA) elemental analyzer. Thin layer chromatography (TLC) was performed on a 0.2 mm pre–coated plates of silica gel 60 F_{254} (Merck).

4.2. Chemistry

4.2.1. Preparation of 2–(3–nitrophenyl)–1,3–dioxolane 3

A mixture of 3–nitrobenzaldehyde **1** (7.5 mmol), ethylene glycol **2** (11.25 mmol) and *p*–toluenesulfonic acid (PTSA) (0.4 mmol) in toluene (25.0 mL) was heated under reflux for 24 h using a Dean–Stark apparatus. After cooling, the crude was extracted with ethyl acetate and NaOH 1 M. The organic layer was dried over anhydrous

magnesium sulfate, filtered and evaporated under reduced pressure. The solid was dried at room temperature and used without further purification.

4.2.1.1. 2–(3–Nitrophenyl)–1,3–dioxolane ***3***. Beige solid; 91% yield; m.p. 53–55 °C. FTIR (ATR) ν (cm^{-1}): 3093 (=C–H), 1620 (C=C), 1535 and 1360 (NO_2), 1098 and 1070 (C–O–C). MS (70 eV) *m/z* (%): 195/196 [M^+] (33/3), 194 (100), 148 (54), 77 (98), 73 (99).

4.2.2. Preparation of 3–(1,3–dioxolan–2–yl)aniline 4

A mixture of the nitro compound **3** (6.5 mmol), hydrazine hydrate (18.5 mmol) and Raney nickel (356 mg) in methanol (25.0 mL) was heated under reflux for 1 h. The hot solution was filtered and evaporated under reduced pressure. After cooling, the oil was filtered through a pad of silica gel for purification.

4.2.2.1. 3–(1,3–Dioxolan–2–yl)aniline ***4***. Yellow oil; 89% yield. FTIR (ATR) ν (cm^{-1}): 3365 and 3226 (NH_2), 1625 (C=C), 1316 (=C–N), 1092 and 1070 (C–O–C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 3.86–4.02 (m, 4H, $H_{4'}$, $H_{5'}$), 5.09 (s, 2H, NH_2), 5.56 (s, 1H, $H_{2'}$), 6.53–6.59 (m, 2H, H_4, H_6), 6.66 (d, J = 1.7 Hz, 1H, H_2), 7.01 (t, J = 7.7 Hz, 1H, H_5). MS (70 eV) *m/z* (%): 165/166 [M^+] (100/11), 164 (49), 120 (26), 93 (40), 73 (23).

4.2.3. Preparation of 3–((7–chloroquinolin–4–yl)amino) benzaldehyde 6

A mixture of the amine **4** (2.75 mmol), and 4,7–dichloroquinoline **5** (2.5 mmol) in ethanol (10.0 mL) was heated under reflux for 3 h. After cooling, HCl 37% (0.5 mL) was added and the solution was stirred at room temperature for 5 min. The solid formed was filtered and washed with ethanol.

4.2.3.1. 3–((7–Chloroquinolin–4–yl)amino)benzaldehyde ***6***. Beige solid; 98% yield; m.p. 195–197 °C. FTIR (ATR) ν (cm^{-1}): 3443 (NH), 3069 (=C–H), 2897 (–(C=O)–H), 1700 (C=O). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 6.90 (d, J = 7.0 Hz, 1H, H_3), 7.80 (t, J = 7.7 Hz, 1H, H_m), 7.83–7.90 (m, 2H, H_6, H_o), 7.96 (d, J = 7.4 Hz, 1H, H_p), 8.01 (s, 1H, $H_{o'}$), 8.22 (d, J = 2.0 Hz, 1H, H_8), 8.56 (d, J = 7.0 Hz, 1H, H_2), 8.96 (d, J = 9.2 Hz, 1H, H_5), 10.08 (s, 1H, CHO), 11.44 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 100.5, 116.2, 119.2, 125.5, 126.4, 127.5, 128.5, 130.9, 131.1, 137.6, 138.0, 138.5, 139.0, 143.6, 154.8, 192.6. MS (70 eV) *m/z* (%): 282/284 [M^+] (100/32), 281 (52), 253 (60), 218 (58), 190 (17).

4.2.4. General procedure for the synthesis of [(7–chloroquinolin–4–yl)amino]chalcones 8a–f

A mixture of the aldehyde **6** (1.00 mmol), the respective acetophenone **7a**–**f** (1.2 mmol) and potassium hydroxide (100 mg) in methanol (10.0 mL) was stirred at room temperature for 12 h. The solid formed was filtered and washed with methanol.

4.2.4.1. (E)–1–(4–Chlorophenyl)–3–(3–((7–chloroquinolin–4–yl) amino)phenyl)prop–2–en–1–one ***8a***. Beige solid; 90% yield; m.p. 210–213 °C. FTIR (ATR) ν (cm^{-1}): 3350 (NH), 3064 (=C–H), 1659 (C=O), 1604 and 1569 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 6.94 (d, J = 5.2 Hz, 1H, H_3), 7.46 (d, J = 7.8 Hz, 1H, H_{Ao}), 7.52 (t, J = 7.7 Hz, 1H, H_{Am}), 7.58 (dd, J = 9.0, 1.9 Hz, 1H, H_6), 7.62 (d, J = 8.5 Hz, 2H, H_{Bm}), 7.69 (d, J = 7.4 Hz, 1H, H_{Ap}), 7.78 (d, J = 15.6 Hz, 1H, $H_{3'}$), 7.91 (s, 2H, H_8, $H_{Ao'}$), 7.95 (d, J = 15.6 Hz, 1H, $H_{2'}$), 8.18 (d, J = 8.5 Hz, 2H, H_{Bo}), 8.43–8.52 (m, 2H, H_2, H_5), 9.31 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 102.1, 118.4, 122.2, 122.9, 124.5, 124.8, 125.0, 125.1, 127.7, 128.9, 130.0, 130.5, 133.9, 135.9, 136.2, 138.2, 140.9, 144.2, 147.9, 149.6, 152.1, 188.1. MS (70 eV) *m/z* (%): 418/420 [M^+] (100/64), 279 (40), 243 (77), 139 (56), 111 (50). Anal. Calcd. For $C_{24}H_{16}Cl_2N_2O$: C, 68.75; H, 3.85; N, 6.68. Found: C, 68.76; H, 3.83; N, 6.65.

4.2.4.2. (E)–1–(4–Bromophenyl)–3–(3–((7–chloroquinolin–4–yl) amino)phenyl)prop–2–en–1–one ***8b***. Beige solid; 87% yield; m.p. 206–208 °C. FTIR (ATR) ν (cm^{-1}): 3354 (NH), 3069 (=C–H), 1659 (C=O), 1603 and 1570 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 6.95 (d, J = 5.3 Hz, 1H, H_3), 7.46 (d, J = 8.0 Hz, 1H, H_{Ao}), 7.53 (t, J = 7.8 Hz, 1H, H_{Am}), 7.59 (dd, J = 9.0, 1.8 Hz, 1H, H_6), 7.70 (d, J = 7.5 Hz, 1H, H_{Ap}), 7.74–7.84 (m, 3H, $H_{3'}$, H_{Bm}), 7.87–7.98 (m, 3H, H_8, $H_{2'}$, $H_{Ao'}$), 8.11 (d, J = 8.4 Hz, 2H, H_{Bo}), 8.43–8.51 (m, 2H, H_2, H_5), 9.15 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 102.1, 118.4, 122.2, 122.9, 124.4, 124.8, 125.0, 125.1, 127.4, 127.7, 130.0, 130.6, 131.9, 133.9, 135.9, 136.5, 141.0, 144.2, 147.9, 149.5, 152.0, 188.3. MS (70 eV) *m/z* (%): 462/464 [M^+] (77/100), 279 (49), 243 (75), 185 (38), 155 (33). Anal. Calcd. For $C_{24}H_{16}BrClN_2O$: C, 62.16; H, 3.48; N, 6.04. Found: C, 62.18; H, 3.51; N, 6.03.

4.2.4.3. (E)–3–(3–((7–Chloroquinolin–4–yl)amino)phenyl)–1–(4–methoxyphenyl)prop–2–en–1–one ***8c***. Yellow solid; 77% yield; m.p. 245–246 °C. FTIR (ATR) ν (cm^{-1}): 3361 (NH), 3009 (=C–H), 1650 (C=O), 1611 and 1573 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 3.87 (s, 3H, OCH_3), 6.95 (d, J = 4.8 Hz, 1H, H_3), 7.08 (d, J = 8.5 Hz, 2H, H_{Bm}), 7.44 (d, J = 7.9 Hz, 1H, H_{Ao}), 7.52 (t, J = 7.7 Hz, 1H, H_{Am}), 7.60 (d, J = 9.1 Hz, 1H, H_6), 7.64–7.77 (m, 2H, $H_{3'}$, H_{Ap}), 7.87 (s, 1H, $H_{Ao'}$), 7.92 (s, 1H, H_8), 7.95 (d, J = 15.8 Hz, 1H, $H_{2'}$), 8.17 (d, J = 8.5 Hz, 2H, H_{Bo}), 8.46 (d, J = 9.1 Hz, 1H, H_5), 8.49 (d, J = 4.8 Hz, 1H, H_2), 9.19 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 55.6, 102.0, 114.0, 118.4, 122.5, 122.8, 124.4, 124.6, 124.8, 125.0, 127.7, 130.0, 130.4, 131.0, 133.9, 136.1, 140.9, 142.8, 148.0, 149.6, 152.1, 163.3, 187.4. MS (70 eV) *m/z* (%): 414/416 [M^+] (97/31), 385 (51), 243 (32), 135 (100), 107 (26). Anal. Calcd. For $C_{25}H_{19}ClN_2O_2$: C, 72.37; H, 4.62; N, 6.75. Found: C, 72.33; H, 4.65; N, 6.74.

4.2.4.4. (E)–3–(3–((7–Chloroquinolin–4–yl)amino)phenyl)–1–(3,4,5–trimethoxyphenyl)prop–2–en–1–one ***8d***. Yellow solid; 74% yield; m.p. 235–237 °C. FTIR (ATR) ν (cm^{-1}): 3341 (NH), 3005 (=C–H), 1647 (C=O), 1612 and 1566 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 3.76 (s, 3H, OCH_3), 3.89 (s, 6H, OCH_3), 6.97 (d, J = 5.1 Hz, 1H, H_3), 7.43 (s, 2H, H_{Bo}), 7.47 (d, J = 7.8 Hz, 1H, H_{Ao}), 7.52 (t, J = 7.7 Hz, 1H, H_{Am}), 7.58 (dd, J = 9.0, 1.4 Hz, 1H, H_6), 7.69–7.81 (m, 2H, $H_{3'}$, H_{Ap}), 7.89 (s, 1H, $H_{Ao'}$), 7.90 (d, J = 1.4 Hz, 1H, H_8), 7.95 (d, J = 15.6 Hz, 1H, $H_{2'}$), 8.48 (d, J = 5.1 Hz, 1H, H_2), 8.51 (d, J = 9.0 Hz, 1H, H_5), 9.33 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 56.3, 60.2, 102.0, 106.3, 118.4, 122.4, 123.0, 124.5, 124.6, 124.8, 124.9, 127.6, 129.9, 132.9, 133.9, 136.0, 141.0, 142.1, 143.6, 147.9, 149.6, 152.0, 152.9, 187.9. MS (70 eV) *m/z* (%): 474/476 [M^+] (81/26), 459 (100), 281 (39), 195 (47), 57 (76). Anal. Calcd. For $C_{27}H_{23}ClN_2O_4$: C, 68.28; H, 4.88; N, 5.90. Found: C, 68.24; H, 4.83; N, 5.92.

4.2.4.5. (E)–3–(3–((7–Chloroquinolin–4–yl)amino)phenyl)–1–(p–tolyl)prop–2–en–1–one ***8e***. Beige solid; 81% yield; m.p. 215–217 °C. FTIR (ATR) ν (cm^{-1}): 3352 (NH), 3014 (=C–H), 1665 (C=O), 1616 and 1565 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 2.39 (s, 3H, CH_3), 6.95 (d, J = 5.1 Hz, 1H, H_3), 7.36 (d, J = 7.9 Hz, 2H, H_{Bm}), 7.46 (d, J = 7.5 Hz, 1H, H_{Ao}), 7.51 (t, J = 7.7 Hz, 1H, H_{Am}), 7.58 (dd, J = 9.0, 2.1 Hz, 1H, H_6), 7.68 (d, J = 7.3 Hz, 1H, H_{Ap}), 7.74 (d, J = 15.6 Hz, 1H, $H_{3'}$), 7.85–7.98 (m, 3H, H_8, $H_{2'}$, $H_{Ao'}$), 8.06 (d, J = 7.9 Hz, 2H, H_{Bo}), 8.44–8.55 (m, 2H, H_2, H_5), 9.29 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 21.2, 102.1, 118.4, 122.6, 122.8, 124.5, 124.6, 124.9, 125.0, 127.7, 128.7, 129.4, 130.0, 133.9, 135.0, 136.0, 140.9, 143.3, 143.7, 147.9, 149.6, 152.1, 188.6. MS (70 eV) *m/z* (%): 398/400 [M^+] (100/32), 243 (33), 221 (36), 119 (58), 91 (74). Anal. Calcd. For $C_{25}H_{19}ClN_2O$: C, 75.28; H, 4.80; N, 7.02. Found: C, 75.28; H, 4.82; N, 7.06.

4.2.4.6. (E)–3–(3–((7–Chloroquinolin–4–yl)amino)phenyl)–1–phenylprop–2–en–1–one ***8f***. Yellow solid; 71% yield; m.p. 220–222 °C. FTIR (ATR) ν (cm^{-1}): 3380 (NH), 3054 (=C–H), 1653 (C=O), 1613 and 1562 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 6.96 (d, J = 5.3 Hz, 1H, H_3), 7.47 (d, J = 7.9 Hz, 1H, H_{Ao}), 7.50–7.60 (m, 4H, H_6, H_{Bm}, H_{Am}), 7.64–7.72 (m, 2H, H_{Bp}, H_{Ap}), 7.76 (d, J = 15.7 Hz, 1H, $H_{3'}$), 7.89 (s, 1H, $H_{Ao'}$), 7.91 (d, J = 2.0 Hz, 1H, H_8), 7.94 (d, J = 15.7 Hz, 1H, $H_{2'}$), 8.15 (d, J = 7.6 Hz, 2H, H_{Bo}), 8.45–8.54 (m, 2H, H_2, H_5), 9.29 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 102.1, 118.4, 122.5, 122.8, 124.5, 124.6, 124.9, 125.0, 127.7, 128.5, 128.8, 130.0, 133.2, 133.9, 135.9, 137.5, 140.9, 143.7, 147.9, 149.6, 152.1, 189.2. MS (70 eV) *m/z* (%): 384/386 [M^+] (100/37), 243 (50), 207 (53), 105 (55), 77 (75). Anal. Calcd. For $C_{24}H_{17}ClN_2O$: C, 74.90; H, 4.45; N, 7.28. Found: C, 74.90; H, 4.49; N, 7.31.

4.2.5. General procedure for the synthesis of 1–(3–aryl–5–(3–((7–chloroquinolin–4–yl)amino)phenyl)–4,5–dihydro–1H–pyrazol–1–yl)ethan–1–ones 9a–f

A mixture of the chalcone **8a**–**f** (0.40 mmol) and hydrazine hydrate (2.00 mmol) in ethanol (1.0 mL) was heated under reflux for 10 min. After cooling, acetic anhydride (2.0 mL) was added and the solution was stirred at room temperature for 10 min. The solid formed was filtered and washed with water and ethanol.

4.2.5.1. 1–(3–(4–Chlorophenyl)–5–(3–((7–chloroquinolin–4–yl)amino)phenyl)–4,5–dihydro–1H–pyrazol–1–yl)ethan–1–one ***9a***. White solid; 86% yield; m.p. 198–199 °C. FTIR (ATR) ν (cm^{-1}): 3352 (NH), 3020 (=C–H), 1672 (C=O), 1603 and 1543 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 2.32 (s, 3H, CH_3), 3.24 (dd, J = 18.2, 4.9 Hz, 1H, H_A), 3.92 (dd, J = 18.2, 12.0 Hz, 1H, H_M), 5.63 (dd, J = 12.0, 4.9 Hz, 1H, H_X), 6.82 (d, J = 7.0 Hz, 1H, H_3), 7.27 (d, J = 7.8 Hz, 1H, H_{Ap}), 7.30 (s, 1H, $H_{Ao'}$), 7.40 (d, J = 8.3 Hz, 1H, H_{Ao}), 7.50–7.56 (m, 3H, H_{Bm}, H_{Am}), 7.80 (d, J = 8.6 Hz, 2H, H_{Bo}), 7.83 (dd, J = 9.1, 1.5 Hz, 1H, H_6), 8.18 (s, 1H, H_8), 8.52 (d, J = 7.0 Hz, 1H, H_2), 8.82 (d, J = 9.1 Hz, 1H, H_5), Not observed (1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 21.7, 41.9, 59.4, 100.3, 116.1, 119.5, 122.2, 123.8, 124.7, 126.1, 127.2, 128.4, 128.9, 129.9, 130.2, 134.9, 137.3, 138.2, 139.4, 143.6, 144.2, 153.2, 154.5, 167.7. MS (70 eV) *m/z* (%): 474/476 [M^+] (58/37), 403 (99), 254 (47), 179 (44), 43 (100). Anal. Calcd. For $C_{26}H_{20}Cl_2N_4O$: C, 65.69; H, 4.24; N, 11.79. Found: C, 65.63; H, 4.25; N, 11.79.

4.2.5.2. 1–(3–(4–Bromophenyl)–5–(3–((7–chloroquinolin–4–yl)amino)phenyl)–4,5–dihydro–1H–pyrazol–1–yl)ethan–1–one ***9b***. White solid; 90% yield; m.p. 211–213 °C. FTIR (ATR) ν (cm^{-1}): 3348 (NH), 3018 (=C–H), 1672 (C=O), 1602 and 1543 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 2.32 (s, 3H, CH_3), 3.24 (dd, J = 18.2, 4.9 Hz, 1H, H_A), 3.92 (dd, J = 18.2, 12.0 Hz, 1H, H_M), 5.63 (dd, J = 12.0, 4.9 Hz, 1H, H_X), 6.83 (d, J = 7.0 Hz, 1H, H_3), 7.26 (d, J = 7.8 Hz, 1H, H_{Ap}), 7.30 (s, 1H, $H_{Ao'}$), 7.40 (d, J = 8.0 Hz, 1H, H_{Ao}), 7.53 (t, J = 7.8 Hz, 1H, H_{Am}), 7.66 (d, J = 8.6 Hz, 2H, H_{Bm}), 7.72 (d, J = 8.6 Hz, 2H, H_{Bo}), 7.83 (d, J = 9.1 Hz, 1H, H_6), 8.17 (d, J = 1.9 Hz, 1H, H_8), 8.52 (d, J = 7.0 Hz, 1H, H_2), 8.82 (d, J = 9.1 Hz, 1H, H_5), Not observed (1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 21.7, 41.9, 59.4, 100.3, 116.1, 119.5, 122.2, 123.7, 123.8, 124.6, 126.1, 127.2, 128.6, 130.1, 130.2, 131.8, 137.3, 138.2, 139.5, 143.6, 144.2, 153.3, 154.4, 167.7. MS (70 eV) *m/z* (%): 518/520 [M^+] (52/66), 478 (25), 449 (100), 254 (32), 43 (72). Anal. Calcd. For $C_{26}H_{20}BrClN_4O$: C, 60.07; H, 3.88; N, 10.78. Found: C, 60.10; H, 3.93; N, 10.85.

4.2.5.3. 1–(5–(3–((7–Chloroquinolin–4–yl)amino)phenyl)–3–(4–methoxyphenyl)–4,5–dihydro–1H–pyrazol–1–yl)ethan–1–one ***9c***. Yellow solid; 80% yield; m.p. 206–208 °C. FTIR (ATR) ν (cm^{-1}): 3351 (NH), 3017 (=C–H), 1679 (C=O), 1604 and 1546 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 2.31 (s, 3H, CH_3), 3.21 (dd, J = 18.0, 4.7 Hz, 1H, H_A), 3.80 (s, 3H, OCH_3), 3.89 (dd, J = 18.0, 11.9 Hz, 1H, H_M), 5.60 (dd, J = 11.9, 4.7 Hz, 1H, H_X), 6.82 (d, J = 7.0 Hz, 1H, H_3), 7.01 (d, J = 8.8 Hz, 2H, H_{Bm}), 7.27 (d, J = 7.9 Hz, 1H, H_{Ap}), 7.29 (s, 1H, $H_{Ao'}$), 7.40 (d, J = 8.1 Hz, 1H, H_{Ao}), 7.53 (t, J = 7.8 Hz, 1H, H_{Am}), 7.72 (d, J = 8.8 Hz, 2H, H_{Bo}), 7.84 (d, J = 9.1 Hz, 1H, H_6), 8.19 (s, 1H, H_8), 8.51 (d, J = 7.0 Hz, 1H, H_2), 8.83 (d, J = 9.1 Hz, 1H, H_5), Not observed (1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 21.7, 42.1, 55.4, 59.0, 100.2, 114.2, 116.0, 119.2, 122.2, 123.5, 123.8, 124.7, 126.1, 127.3, 128.3, 130.2, 137.2, 138.3, 139.1, 143.3, 144.5, 154.0, 154.7, 161.0, 167.3. MS (70 eV) *m/z* (%): 470/472 [M^+] (59/22), 428 (31), 399 (100), 175 (32), 43 (60). Anal. Calcd. For $C_{27}H_{23}ClN_4O_2$: C, 68.86; H, 4.92; N, 11.90. Found: C, 68.90; H, 4.89; N, 11.90.

4.2.5.4. 1–(5–(3–((7–Chloroquinolin–4–yl)amino)phenyl)–3–(3,4,5–trimethoxyphenyl)–4,5–dihydro–1H–pyrazol–1–yl)ethan–1–one ***9d***. Yellow solid; 77% yield; m.p. 210–212 °C. FTIR (ATR) ν (cm^{-1}): 3383 (NH), 3028 (=C–H), 1674 (C=O), 1602 and 1568 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 2.31 (s, 3H, CH_3), 3.21 (dd, J = 18.0, 4.7 Hz, 1H, H_A), 3.74 (s, 3H, OCH_3), 3.85–3.92 (m, 7H, OCH_3, H_M), 5.60 (dd, J = 11.8, 4.7 Hz, 1H, H_X), 6.81 (d, J = 7.0 Hz, 1H, H_3), 7.03 (s, 2H, H_{Bo}), 7.26 (d, J = 7.9 Hz, 1H, H_{Ap}), 7.31 (s, 1H, $H_{Ao'}$), 7.40 (d, J = 8.0 Hz, 1H, H_{Ao}), 7.53 (t, J = 7.8 Hz, 1H, H_{Am}), 7.84 (d, J = 9.0 Hz, 1H, H_6), 8.17 (s, 1H, H_8), 8.51 (d, J = 7.0 Hz, 1H, H_2), 8.82 (d, J = 9.0 Hz, 1H, H_5), Not observed (1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 21.7, 42.1, 56.4, 59.0, 60.5, 100.2, 114.2, 116.5, 119.2, 122.3, 123.5, 123.7, 124.9, 126.1, 127.5, 128.3, 130.2, 137.3, 138.3, 139.3, 143.4, 144.5, 154.0, 154.6, 161.0, 167.3. MS (70 eV) *m/z* (%): 530/532 [M^+] (100/37), 499 (83), 254 (46), 239 (24), 43 (89). Anal. Calcd. For $C_{29}H_{27}ClN_4O_4$: C, 65.60; H, 5.13; N, 10.55. Found: C, 65.59; H, 5.10; N, 10.57.

4.2.5.5. 1–(5–(3–((7–Chloroquinolin–4–yl)amino)phenyl)–3–(p–tolyl)–4,5–dihydro–1H–pyrazol–1–yl)ethan–1–one ***9e***. White solid; 78% yield; m.p. 199–201 °C. FTIR (ATR) ν (cm^{-1}): 3338 (NH), 3016 (=C–H), 1679 (C=O), 1606 and 1545 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 2.32 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 3.21 (dd, J = 18.1, 4.8 Hz, 1H, H_A), 3.90 (dd, J = 18.1, 11.9 Hz, 1H, H_M), 5.61 (dd, J = 11.9, 4.8 Hz, 1H, H_X), 6.82 (d, J = 7.0 Hz, 1H, H_3), 7.23–7.28 (m, 3H, H_{Bm}, H_{Ap}), 7.29 (s, 1H, $H_{Ao'}$), 7.40 (d, J = 8.1 Hz, 1H, H_{Ao}), 7.53 (t, J = 7.8 Hz, 1H, H_{Am}), 7.67 (d, J = 8.1 Hz, 2H, H_{Bo}), 7.84 (dd, J = 9.1, 1.9 Hz, 1H, H_6), 8.19 (d, J = 1.9 Hz, 1H, H_8), 8.51 (d, J = 7.0 Hz, 1H, H_2), 8.83 (d, J = 9.1 Hz, 1H, H_5), Not observed (1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 21.0, 21.7, 42.0, 59.1, 100.2, 116.0, 119.3, 122.2, 123.8, 124.7, 126.1, 126.6, 127.3, 128.2, 129.3, 130.2, 137.2, 138.3, 139.2, 140.2, 143.4, 144.4, 154.2, 154.6, 167.5. MS (70 eV) *m/z* (%): 454/456 [M^+] (48/17), 383 (63), 254 (13), 159 (21), 43 (100). Anal. Calcd. For $C_{27}H_{23}ClN_4O$: C, 71.28; H, 5.10; N, 12.31. Found: C, 71.28; H, 5.15; N, 12.33.

4.2.5.6. 1–(5–(3–((7–Chloroquinolin–4–yl)amino)phenyl)–3–phenyl–4,5–dihydro–1H–pyrazol–1–yl)ethan–1–one ***9f***. White solid; 75% yield; m.p. 193–195 °C. FTIR (ATR) ν (cm^{-1}): 3340 (NH), 3017 (=C–H), 1674 (C=O), 1601 and 1542 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 2.33 (s, 3H, CH_3), 3.24 (dd, J = 18.1, 4.8 Hz, 1H, H_A), 3.93 (dd, J = 18.1, 12.0 Hz, 1H, H_M), 5.63 (dd, J = 11.9, 4.8 Hz, 1H, H_X), 6.82 (d, J = 7.0 Hz, 1H, H_3), 7.28 (d, J = 7.7 Hz, 1H, H_{Ap}), 7.31 (s, 1H, $H_{Ao'}$), 7.40 (d, J = 8.1 Hz, 1H, H_{Ao}), 7.44–7.50 (m, 3H, H_{Bm}, H_{Bp}), 7.54 (t, J = 7.8 Hz, 1H, H_{Am}), 7.76–7.81 (m, 2H, H_{Bo}), 7.84 (d, J = 8.4 Hz, 1H, H_6), 8.19 (s, 1H, H_8), 8.51 (d, J = 7.0 Hz, 1H, H_2), 8.83 (d, J = 8.4 Hz, 1H, H_5), Not observed (1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 21.7, 42.0, 59.2, 100.3, 116.0, 119.2, 122.3, 123.9, 124.7, 126.1, 126.6, 127.3, 128.8, 130.2, 130.4, 131.0, 137.2, 138.3, 139.1, 143.4, 144.4, 154.2, 154.7, 167.6. MS (70 eV) *m/z*

(%): 440/442 [M^+] (28/10), 369 (50), 254 (10), 145 (17), 43 (100). Anal. Calcd. For $C_{26}H_{21}ClN_4O$: C, 70.82; H, 4.80; N, 12.71. Found: C, 70.81; H, 4.79; N, 12.72.

4.2.6. General procedure for the synthesis of 3–aryl–5–(3–((7–chloroquinolin–4–yl)amino)phenyl)–4,5–dihydro–1H–pyrazole–1–carbaldehydes 10a–f

A mixture of the chalcone **8a**–**f** (0.40 mmol) and hydrazine hydrate (2.00 mmol) in ethanol (1.0 mL) was heated under reflux for 10 min. After cooling, formic acid (2.0 mL) was added and the solution was stirred at room temperature for 10 min. The solid formed was filtered and washed with water and ethanol.

4.2.6.1. 3–(4–Chlorophenyl)–5–(3–((7–chloroquinolin–4–yl)amino)phenyl)–4,5–dihydro–1H–pyrazole–1–carbaldehyde ***10a***. White solid; 88% yield; m.p. 139–141 °C. FTIR (ATR) ν (cm^{-1}): 3340 (NH), 3021 (=C–H), 1675 (C=O), 1600 and 1539 (C=N and C=C). ^1H NMR (400 MHz, DMSO–d_6) δ ppm 3.30 (dd, J = 18.2, 4.9 Hz, 1H, H_A), 3.94 (dd, J = 18.2, 11.8 Hz, 1H, H_M), 5.58 (dd, J = 11.8, 4.9 Hz, 1H, H_X), 6.96 (d, J = 5.4 Hz, 1H, H_3), 7.05 (d, J = 7.7 Hz, 1H, H_{Ap}), 7.22 (s, 1H, $H_{Ao'}$), 7.30 (d, J = 8.0 Hz, 1H, H_{Ao}), 7.40 (t, J = 7.8 Hz, 1H, H_{Am}), 7.50–7.59 (m, 3H, H_6, H_{Bm}), 7.80 (d, J = 8.6 Hz, 2H, H_{Bo}), 7.89 (d, J = 2.2 Hz, 1H, H_8), 8.40 (d, J = 9.0 Hz, 1H, H_5), 8.43 (d, J = 5.4 Hz, 1H, H_2), 8.93 (s, 1H, CHO), Not observed (1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 42.2, 58.6, 102.0, 118.3, 119.4, 121.1, 121.3, 124.5, 125.1, 127.3, 128.5, 128.9, 129.6, 129.8, 134.1, 135.2, 140.5, 142.6, 147.9, 149.2, 151.5, 155.3, 159.8. MS (70 eV) m/z (%): 460/462 [M^+] (100/68), 431 (17), 403 (52), 254 (58), 218 (25). Anal. Calcd. For $C_{25}H_{18}Cl_2N_4O$: C, 65.09; H, 3.93; N, 12.14. Found: C, 65.05; H, 3.94; N, 12.14.

4.2.6.2. 3–(4–Bromophenyl)–5–(3–((7–chloroquinolin–4–yl)amino)phenyl)–4,5–dihydro–1H–pyrazole–1–carbaldehyde ***10b***. White solid; 86% yield; m.p. 136–138 °C. FTIR (ATR) ν (cm^{-1}): 3336 (NH), 3019 (=C–H), 1675 (C=O), 1602 and 1544 (C=N and C=C). ^1H NMR (400 MHz, DMSO–d_6) δ ppm 3.30 (dd, J = 18.2, 4.9 Hz, 1H, H_A), 3.95 (dd, J = 18.2, 11.9 Hz, 1H, H_M), 5.58 (dd, J = 11.9, 4.9 Hz, 1H, H_X), 6.94 (d, J = 5.6 Hz, 1H, H_3), 7.09 (d, J = 7.6 Hz, 1H, H_{Ap}), 7.24 (s, 1H, $H_{Ao'}$), 7.32 (d, J = 8.5 Hz, 1H, H_{Ao}), 7.43 (t, J = 7.8 Hz, 1H, H_{Am}), 7.61 (dd, J = 9.0, 1.9 Hz, 1H, H_6), 7.68 (d, J = 8.6 Hz, 2H, H_{Bm}), 7.73 (d, J = 8.6 Hz, 2H, H_{Bo}), 7.92 (d, J = 1.9 Hz, 1H, H_8), 8.42–8.48 (m, 2H, H_2, H_5), 8.93 (s, 1H, CHO), Not observed (1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 42.1, 58.6, 101.7, 118.0, 119.9, 121.6, 121.9, 124.0, 124.7, 125.4, 126.2, 128.7, 129.9, 130.0, 131.8, 134.7, 140.1, 142.7, 147.7, 148.9, 150.4, 155.4, 159.8. MS (70 eV) m/z (%): 504/506 [M^+] (45/61), 477 (15), 449 (100), 253 (45), 218 (22). Anal. Calcd. For $C_{25}H_{18}BrClN_4O$: C, 59.37; H, 3.59; N, 11.08. Found: C, 59.38; H, 3.52; N, 11.14.

4.2.6.3. 5–(3–((7–Chloroquinolin–4–yl)amino)phenyl)–3–(4–methoxyphenyl)–4,5–dihydro–1H–pyrazole–1–carbaldehyde ***10c***. Yellow solid; 79% yield; m.p. 104–106 °C. FTIR (ATR) ν (cm^{-1}): 3339 (NH), 3018 (=C–H), 1658 (C=O), 1607 and 1544 (C=N and C=C). ^1H NMR (400 MHz, DMSO–d_6) δ ppm 3.27 (dd, J = 18.1, 4.7 Hz, 1H, H_A), 3.80 (s, 3H, OCH_3), 3.93 (dd, J = 18.1, 11.7 Hz, 1H, H_M), 5.57 (dd, J = 11.7, 4.7 Hz, 1H, H_X), 6.91 (d, J = 6.1 Hz, 1H, H_3), 7.02 (d, J = 8.9 Hz, 2H, H_{Bm}), 7.16 (d, J = 7.7 Hz, 1H, H_{Ap}), 7.26 (s, 1H, $H_{Ao'}$), 7.35 (d, J = 8.0 Hz, 1H, H_{Ao}), 7.47 (t, J = 7.8 Hz, 1H, H_{Am}), 7.68 (dd, J = 9.1, 2.1 Hz, 1H, H_6), 7.74 (d, J = 8.9 Hz, 2H, H_{Bo}), 7.98 (d, J = 2.1 Hz, 1H, H_8), 8.47 (d, J = 6.1 Hz, 1H, H_2), 8.55 (d, J = 9.1 Hz, 1H, H_5), 8.90 (s, 1H, CHO), Not observed (1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 42.3, 55.4, 58.2, 101.3, 114.3, 117.4, 120.6, 122.3, 122.9, 123.1, 124.0, 125.1, 126.0, 128.5, 130.0, 135.9, 139.2, 143.1, 148.3, 150.7, 155.9, 159.5, 161.1, 163.0. MS (70 eV) m/z (%): 456/458 [M^+] (73/26), 427 (23), 425 (16), 399 (100), 254 (32). Anal. Calcd. For $C_{26}H_{21}ClN_4O_2$: C, 68.34; H, 4.63; N, 12.26. Found: C, 68.38; H, 4.64; N, 12.27.

4.2.6.4. 5–(3–((7–Chloroquinolin–4–yl)amino)phenyl)–3–(3,4,5–trimethoxyphenyl)–4,5–dihydro–1H–pyrazole–1–carbaldehyde ***10d***. Yellow solid; 77% yield; m.p. 108–110 °C. FTIR (ATR) ν (cm^{-1}): 3371 (NH), 3026 (=C–H), 1684 (C=O), 1609 and 1548 (C=N and C=C). ^1H NMR (400 MHz, DMSO–d_6) δ ppm 3.37 (dd, J = 18.2, 4.6 Hz, 1H, H_A), 3.70 (s, 3H, OCH_3), 3.82 (s, 6H, OCH_3), 3.93 (dd, J = 18.2, 11.8 Hz, 1H, H_M), 5.59 (dd, J = 11.8, 4.6 Hz, 1H, H_X), 6.94 (d, J = 5.8 Hz, 1H, H_3), 7.07 (s, 2H, H_{Bo}), 7.11 (d, J = 7.7 Hz, 1H, H_{Ap}), 7.24 (s, 1H, $H_{Ao'}$), 7.33 (d, J = 8.0 Hz, 1H, H_{Ao}), 7.45 (t, J = 7.8 Hz, 1H, H_{Am}), 7.63 (dd, J = 9.0, 2.1 Hz, 1H, H_6), 7.93 (d, J = 2.1 Hz, 1H, H_8), 8.44–8.50 (m, 2H, H_2, H_5), 8.93 (s, 1H, CHO), Not observed (1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 42.4, 56.1, 58.4, 60.2, 101.6, 104.2, 117.8, 120.0, 121.8, 122.1, 124.8, 125.6, 126.1, 130.0, 135.1, 135.9, 139.7, 139.9, 142.9, 149.4, 149.9, 153.1, 156.2, 159.6, 163.0. MS (70 eV) m/z (%): 516/518 [M^+] (57/20), 501 (100), 469 (30), 310 (58), 254 (28). Anal. Calcd. For $C_{28}H_{25}ClN_4O_4$: C, 65.05; H, 4.87; N, 10.84. Found: C, 65.00; H, 4.89; N, 10.86.

4.2.6.5. 5–(3–((7–Chloroquinolin–4–yl)amino)phenyl)–3–(p–tolyl)–4,5–dihydro–1H–pyrazole–1–carbaldehyde ***10e***. White solid; 75% yield; m.p. 120–122 °C. FTIR (ATR) ν (cm^{-1}): 3326 (NH), 3018 (=C–H), 1674 (C=O), 1604 and 1543 (C=N and C=C). ^1H NMR (400 MHz, DMSO–d_6) δ ppm 2.34 (s, 3H, CH_3), 3.26 (dd, J = 18.1, 4.8 Hz, 1H, H_A), 3.93 (dd, J = 18.1, 11.8 Hz, 1H, H_M), 5.57 (dd, J = 11.8, 4.8 Hz, 1H, H_X), 6.92 (d, J = 5.8 Hz, 1H, H_3), 7.11 (d, J = 7.7 Hz, 1H, H_{Ap}), 7.25 (s, 1H, $H_{Ao'}$), 7.27 (d, J = 8.1 Hz, 2H, H_{Bm}), 7.33 (d, J = 8.0 Hz, 1H, H_{Ao}), 7.44 (t, J = 7.8 Hz, 1H, H_{Am}), 7.63 (dd, J = 9.1, 2.0 Hz, 1H, H_6), 7.68 (d, J = 8.1 Hz, 2H, H_{Bo}), 7.95 (d, J = 2.0 Hz, 1H, H_8), 8.45 (d, J = 5.8 Hz, 1H, H_2), 8.50 (d, J = 9.1 Hz, 1H, H_5), 8.91 (s, 1H, CHO), Not observed (1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 21.0, 42.3, 58.3, 102.0, 117.7, 120.2, 121.9, 122.3, 124.9, 125.2, 125.7, 126.7, 127.9, 129.4, 129.9, 135.2, 139.7, 140.5, 142.9, 146.5, 149.4, 149.7, 156.2, 159.6. MS (70 eV) m/z (%): 440/442 [M^+] (100/33), 405 (18), 383 (37), 254 (23), 159 (37). Anal. Calcd. For $C_{26}H_{21}ClN_4O$: C, 70.82; H, 4.80; N, 12.71. Found: C, 70.80; H, 4.82; N, 12.74.

4.2.6.6. 5–(3–((7–Chloroquinolin–4–yl)amino)phenyl)–3–phenyl–4,5–dihydro–1H–pyrazole–1–carbaldehyde ***10f***. White solid; 81% yield; m.p. 141–143 °C. FTIR (ATR) ν (cm^{-1}): 3328 (NH), 3018 (=C–H), 1680 (C=O), 1606 and 1536 (C=N and C=C). ^1H NMR (400 MHz, DMSO–d_6) δ ppm 3.30 (dd, J = 18.2, 4.8 Hz, 1H, H_A), 3.96 (dd, J = 18.2, 11.8 Hz, 1H, H_M), 5.58 (dd, J = 11.8, 4.8 Hz, 1H, H_X), 6.94 (d, J = 5.7 Hz, 1H, H_3), 7.11 (d, J = 7.7 Hz, 1H, H_{Ap}), 7.25 (s, 1H, $H_{Ao'}$), 7.32 (d, J = 8.0 Hz, 1H, H_{Ao}), 7.43 (t, J = 7.9 Hz, 1H, H_{Am}), 7.46–7.50 (m, 3H, H_{Bm}, H_{Bp}), 7.61 (dd, J = 9.1, 2.1 Hz, 1H, H_6), 7.76–7.84 (m, 2H, H_{Bo}), 7.93 (d, J = 2.1 Hz, 1H, H_8), 8.44 (d, J = 5.7 Hz, 1H, H_2), 8.47 (d, J = 9.1 Hz, 1H, H_5), 8.93 (s, 1H, CHO), Not observed (1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 42.2, 58.4, 101.6, 117.9, 120.0, 121.7, 122.1, 124.8, 125.5, 125.8, 126.7, 128.8, 129.9, 130.6, 130.7, 134.9, 139.9, 142.8, 147.2, 149.2, 150.0, 156.2, 159.7. MS (70 eV) m/z (%): 426/428 [M^+] (100/36), 397 (18), 369 (45), 254 (36), 218 (15). Anal. Calcd. For $C_{25}H_{19}ClN_4O$: C, 70.34; H, 4.49; N, 13.12. Found: C, 70.30; H, 4.50; N, 13.10.

4.2.7. General procedure for the synthesis of N–(3–(3–aryl–1–phenyl–4,5–dihydro–1H–pyrazol–5–yl)phenyl)–7–chloroquinolin–4–amines 11a–f

A mixture of the chalcone **8a**–**f** (1.00 mmol) and phenylhydrazine (2.00 mmol) in methanol (10.0 mL) was heated under reflux for 3 h. After cooling, the solid formed was filtered and

washed with water and methanol.

4.2.7.1. 7–Chloro–N–(3–(3–(4–chlorophenyl)–1–phenyl–4,5–dihydro–1H–pyrazol–5–yl)phenyl)quinolin–4–amine ***11a***. White solid; 82% yield; m.p. 194–196 °C. FTIR (ATR) *ν* (cm^{-1}): 3347 (NH), 3022 (=C–H), 1602 and 1540 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) *δ* ppm 3.19 (dd, *J* = 17.9, 5.8 Hz, 1H, H_A), 3.92 (dd, *J* = 17.9, 12.3 Hz, 1H, H_M), 5.57 (dd, *J* = 12.3, 5.8 Hz, 1H, H_X), 6.71 (d, *J* = 5.3 Hz, 1H, H_3), 6.78 (t, *J* = 7.2 Hz, 1H, H_{Cp}), 7.05 (d, *J* = 8.2 Hz, 2H, H_{Co}), 7.12 (d, *J* = 7.6 Hz, 1H, H_{Ap}), 7.16–7.30 (m, 4H, H_{Ao}, $H_{Ao'}$, H_{Cm}), 7.40 (t, *J* = 7.8 Hz, 1H, H_{Am}), 7.46 (d, *J* = 8.5 Hz, 2H, H_{Bm}), 7.51 (dd, *J* = 9.1, 1.8 Hz, 1H, H_6), 7.74 (d, *J* = 8.5 Hz, 2H, H_{Bo}), 7.86 (d, *J* = 1.8 Hz, 1H, H_8), 8.28 (d, *J* = 5.3 Hz, 1H, H_2), 8.37 (d, *J* = 9.1 Hz, 1H, H_5), 9.13 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) *δ* ppm 42.4, 58.7, 102.0, 114.5, 118.2, 119.4, 121.3, 121.5, 124.9, 125.3, 127.1, 127.4, 128.7, 128.8, 129.9, 130.1, 132.6, 134.1, 135.3, 140.4, 143.0, 148.2, 149.3, 151.5, 155.1, 159.4. MS (70 eV) *m/z* (%): 508/510 [M^+] (73/49), 254 (57), 111 (14), 91 (100), 57 (27). Anal. Calcd. For $C_{30}H_{22}Cl_2N_4$: C, 70.73; H, 4.35; N, 11.00. Found: C, 70.74; H, 4.34; N, 11.01.

4.2.7.2. N–(3–(3–(4–Bromophenyl)–1–phenyl–4,5–dihydro–1H–pyrazol–5–yl)phenyl)–7–chloroquinolin–4–amine ***11b***. White solid; 79% yield; m.p. 185–187 °C. FTIR (ATR) *ν* (cm^{-1}): 3343 (NH), 3020 (=C–H), 1604 and 1545 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) *δ* ppm 3.19 (dd, *J* = 17.9, 5.8 Hz, 1H, H_A), 3.93 (dd, *J* = 17.9, 12.4 Hz, 1H, H_M), 5.57 (dd, *J* = 12.4, 5.8 Hz, 1H, H_X), 6.71 (d, *J* = 5.6 Hz, 1H, H_3), 6.78 (t, *J* = 7.2 Hz, 1H, H_{Cp}), 7.03 (d, *J* = 8.2 Hz, 2H, H_{Co}), 7.09 (d, *J* = 7.6 Hz, 1H, H_{Ap}), 7.16–7.25 (m, 3H, $H_{Ao'}$, H_{Cm}), 7.35 (d, *J* = 8.5 Hz, 1H, H_{Ao}), 7.41 (t, *J* = 7.8 Hz, 1H, H_{Am}), 7.60 (dd, *J* = 9.0, 1.9 Hz, 1H, H_6), 7.69 (d, *J* = 8.6 Hz, 2H, H_{Bm}), 7.73 (d, *J* = 8.6 Hz, 2H, H_{Bo}), 7.89 (d, *J* = 1.9 Hz, 1H, H_8), 8.43–8.46 (m, 2H, H_2, H_5), 9.12 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) *δ* ppm 41.9, 58.6, 101.5, 114.5, 117.8, 120.3, 121.8, 122.1, 124.0, 124.7, 125.3, 126.5, 127.1, 128.5, 130.0, 130.4, 131.6, 132.6, 134.9, 140.0, 142.6, 147.5, 148.9, 150.7, 155.5, 159.4. MS (70 eV) *m/z* (%): 552/554 [M^+] (86/100), 299 (10), 254 (42), 91 (53), 77 (10). Anal. Calcd. For $C_{30}H_{22}BrClN_4$: C, 65.05; H, 4.00; N, 10.12. Found: C, 65.11; H, 4.03; N, 10.14.

4.2.7.3. 7–Chloro–N–(3–(3–(4–methoxyphenyl)–1–phenyl–4,5–dihydro–1H–pyrazol–5–yl)phenyl)quinolin–4–amine ***11c***. White solid; 69% yield; m.p. 153–155 °C. FTIR (ATR) *ν* (cm^{-1}): 3347 (NH), 3019 (=C–H), 1609 and 1545 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) *δ* ppm 3.16 (dd, *J* = 17.8, 5.6 Hz, 1H, H_A), 3.81 (s, 3H, OCH_3), 3.91 (dd, *J* = 17.8, 12.2 Hz, 1H, H_M), 5.56 (dd, *J* = 12.2, 5.6 Hz, 1H, H_X), 6.71 (d, *J* = 6.1 Hz, 1H, H_3), 6.80 (t, *J* = 7.2 Hz, 1H, H_{Cp}), 7.00 (d, *J* = 8.9 Hz, 2H, H_{Bm}), 7.07 (d, *J* = 8.2 Hz, 2H, H_{Co}), 7.16 (d, *J* = 7.7 Hz, 1H, H_{Ap}), 7.19–7.28 (m, 3H, $H_{Ao'}$, H_{Cm}), 7.38–7.45 (m, 2H, H_{Ao}, H_{Am}), 7.68 (dd, *J* = 9.1, 2.1 Hz, 1H, H_6), 7.74 (d, *J* = 8.9 Hz, 2H, H_{Bo}), 7.98 (d, *J* = 2.1 Hz, 1H, H_8), 8.48 (d, *J* = 6.1 Hz, 1H, H_2), 8.53 (d, *J* = 9.1 Hz, 1H, H_5), 9.14 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) *δ* ppm 42.3, 55.8, 58.4, 101.1, 114.4, 114.5, 117.5, 120.6, 122.1, 122.8, 123.4, 124.1, 125.1, 126.3, 126.7, 128.6, 130.0, 132.5, 135.9, 139.1, 143.1, 148.1, 150.7, 156.1, 159.4, 160.8, 162.7. MS (70 eV) *m/z* (%): 504/506 [M^+] (100/42), 254 (27), 91 (53), 77 (8), 64 (7). Anal. Calcd. For $C_{31}H_{25}ClN_4O$: C, 73.73; H, 4.99; N, 11.09. Found: C, 73.74; H, 5.01; N, 11.09.

4.2.7.4. 7–Chloro–N–(3–(1–phenyl–3–(3,4,5–trimethoxyphenyl)–4,5–dihydro–1H–pyrazol–5–yl)phenyl)quinolin–4–amine ***11d***. White solid; 65% yield; m.p. 182–185 °C. FTIR (ATR) *ν* (cm^{-1}): 3378 (NH), 3030 (=C–H), 1611 and 1549 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) *δ* ppm 3.26 (dd, *J* = 17.9, 5.5 Hz, 1H, H_A), 3.70 (s, 3H, OCH_3), 3.82 (s, 6H, OCH_3), 3.91 (dd, *J* = 17.9, 12.3 Hz, 1H, H_M), 5.58 (dd, *J* = 12.3, 5.5 Hz, 1H, H_X), 6.74 (d, *J* = 5.8 Hz, 1H, H_3), 6.77 (t, *J* = 7.2 Hz, 1H, H_{Cp}), 7.05–7.07 (m, 4H, H_{Bo}, H_{Co}), 7.13 (d, *J* = 7.7 Hz, 1H, H_{Ap}), 7.17–7.25 (m, 3H, $H_{Ao'}$, H_{Cm}), 7.34 (d, *J* = 8.0 Hz, 1H, H_{Ao}), 7.42 (t, *J* = 7.8 Hz, 1H, H_{Am}), 7.62 (dd, *J* = 9.0, 2.1 Hz, 1H, H_6), 7.96 (d, *J* = 2.1 Hz, 1H, H_8), 8.45–8.54 (m, 2H, H_2, H_5), 9.14 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) *δ* ppm 42.3, 56.1, 58.2, 60.4, 101.8, 104.3, 114.7, 117.6, 120.0, 121.9, 122.0, 124.8, 125.2, 126.1, 127.1, 129.8, 132.7, 135.1, 135.7, 139.6, 140.2, 143.1, 149.6, 150.1, 152.7, 156.2, 159.5, 163.1. MS (70 eV) *m/z* (%): 564/566 [M^+] (100/40), 549 (25), 254 (10), 91 (9), 77 (5). Anal. Calcd. For $C_{33}H_{29}ClN_4O_3$: C, 70.14; H, 5.17; N, 9.92. Found: C, 70.17; H, 5.12; N, 9.91.

4.2.7.5. 7–Chloro–N–(3–(1–phenyl–3–(p–tolyl)–4,5–dihydro–1H–pyrazol–5–yl)phenyl)quinolin–4–amine ***11e***. White solid; 73% yield; m.p. 189–191 °C. FTIR (ATR) *ν* (cm^{-1}): 3333 (NH), 3022 (=C–H), 1606 and 1544 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) *δ* ppm 2.37 (s, 3H, CH_3), 3.15 (dd, *J* = 17.8, 5.7 Hz, 1H, H_A), 3.91 (dd, *J* = 17.8, 12.3 Hz, 1H, H_M), 5.56 (dd, *J* = 12.3, 5.7 Hz, 1H, H_X), 6.74 (d, *J* = 5.8 Hz, 1H, H_3), 6.80 (t, *J* = 7.2 Hz, 1H, H_{Cp}), 7.07 (d, *J* = 8.2 Hz, 2H, H_{Co}), 7.10 (d, *J* = 7.7 Hz, 1H, H_{Ap}), 7.17–7.28 (m, 5H, $H_{Ao'}$, H_{Bm}, H_{Cm}), 7.33 (d, *J* = 8.0 Hz, 1H, H_{Ao}), 7.42 (t, *J* = 7.8 Hz, 1H, H_{Am}), 7.65–7.70 (m, 3H, H_6, H_{Bo}), 7.95 (d, *J* = 2.0 Hz, 1H, H_8), 8.47 (d, *J* = 5.8 Hz, 1H, H_2), 8.52 (d, *J* = 9.1 Hz, 1H, H_5), 9.12 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) *δ* ppm 20.9, 42.5, 58.2, 102.0, 114.5, 117.6, 120.2, 121.8, 122.5, 124.5, 125.1, 125.6, 126.5, 127.1, 127.7, 129.7, 129.9, 132.6, 135.1, 139.4, 140.4, 143.0, 146.6, 149.6, 149.8, 155.9, 159.6. MS (70 eV) *m/z* (%): 488/490 [M^+] (4/1), 254 (4), 81 (61), 69 (100), 57 (66). Anal. Calcd. For $C_{31}H_{25}ClN_4$: C, 76.14; H, 5.15; N, 11.46. Found: C, 76.09; H, 5.14; N, 11.52.

4.2.7.6. 7–Chloro–N–(3–(1,3–diphenyl–4,5–dihydro–1H–pyrazol–5–yl)phenyl)quinolin–4–amine ***11f***. White solid; 68% yield; m.p. 144–146 °C. FTIR (ATR) *ν* (cm^{-1}): 3335 (NH), 3019 (=C–H), 1608 and 1537 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) *δ* ppm 3.19 (dd, *J* = 17.9, 5.7 Hz, 1H, H_A), 3.94 (dd, *J* = 17.9, 12.3 Hz, 1H, H_M), 5.57 (dd, *J* = 12.3, 5.7 Hz, 1H, H_X), 6.73 (d, *J* = 5.7 Hz, 1H, H_3), 6.76 (t, *J* = 7.2 Hz, 1H, H_{Cp}), 7.07 (d, *J* = 8.2 Hz, 2H, H_{Co}), 7.10 (d, *J* = 7.7 Hz, 1H, H_{Ap}), 7.15–7.26 (m, 3H, $H_{Ao'}$, H_{Cm}), 7.34 (d, *J* = 8.0 Hz, 1H, H_{Ao}), 7.45–7.47 (m, 4H, H_{Am}, H_{Bm}, H_{Bp}), 7.62 (dd, *J* = 9.1, 2.1 Hz, 1H, H_6), 7.74–7.82 (m, 2H, H_{Bo}), 7.95 (d, *J* = 2.1 Hz, 1H, H_8), 8.47–8.48 (m, 2H, H_2, H_5), 9.10 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) *δ* ppm 42.4, 58.5, 101.2, 114.4, 117.6, 119.8, 121.8, 121.9, 124.9, 125.1, 126.1, 126.5, 127.5, 128.6, 129.9, 130.6, 130.7, 132.5, 135.0, 139.9, 142.9, 147.4, 149.2, 150.0, 156.4, 159.1. MS (70 eV) *m/z* (%): 474/476 [M^+] (22/8), 371 (66), 254 (20), 69 (85), 57 (100). Anal. Calcd. For $C_{30}H_{23}ClN_4$: C, 75.86; H, 4.88; N, 11.80. Found: C, 75.85; H, 4.86; N, 11.81.

4.2.8. General procedure for the synthesis of N–(3–(3–aryl–1–(4–chlorophenyl)–4,5–dihydro–1H–pyrazol–5–yl)phenyl)–7–chloroquinolin–4–amines 12a–f

A mixture of the chalcone **8a**–**f** (1.00 mmol) and 4–chlorophenylhydrazine hydrochloride (2.00 mmol) in methanol (10.0 mL) was heated under reflux for 3 h. After cooling, the solid formed was filtered and washed with water and methanol.

4.2.8.1. N–(3–(1,3–Bis(4–chlorophenyl)–4,5–dihydro–1H–pyrazol–5–yl)phenyl)–7–chloroquinolin–4–amine ***12a***. Yellow solid; 90% yield; m.p. 237–239 °C. FTIR (ATR) *ν* (cm^{-1}): 3345 (NH), 3023 (=C–H), 1600 and 1541 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) *δ* ppm

3.25 (dd, J = 17.7, 6.1 Hz, 1H, H_A), 3.99 (dd, J = 17.7, 12.3 Hz, 1H, H_M), 5.64 (dd, J = 12.3, 6.1 Hz, 1H, H_X), 6.70 (d, J = 6.9 Hz, 1H, H_3), 7.03 (d, J = 8.9 Hz, 2H, H_{Co}), 7.24 (d, J = 8.9 Hz, 2H, H_{Cm}), 7.32–7.38 (m, 2H, H_{Ap}, $H_{Ao'}$), 7.41 (d, J = 8.5 Hz, 1H, H_{Ao}), 7.48 (d, J = 8.5 Hz, 2H, H_{Bm}), 7.56 (t, J = 8.0 Hz, 1H, H_{Am}), 7.75 (d, J = 8.5 Hz, 2H, H_{Bo}), 7.81 (dd, J = 9.1, 2.0 Hz, 1H, H_6), 8.17 (d, J = 2.0 Hz, 1H, H_8), 8.42 (d, J = 6.9 Hz, 1H, H_2), 8.80 (d, J = 9.1 Hz, 1H, H_5), 11.08 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 42.2, 58.9, 101.9, 114.3, 117.8, 119.1, 121.0, 121.7, 124.7, 125.7, 127.0, 127.4, 128.5, 129.0, 130.0, 130.1, 132.6, 134.4, 135.3, 140.0, 143.1, 148.4, 149.5, 151.4, 155.2, 159.4. MS (70 eV) m/z (%): 542/544 [M^+] (82/80), 432 (45), 403 (100), 254 (67), 91 (63). Anal. Calcd. For $C_{30}H_{21}Cl_3N_4$: C, 66.25; H, 3.89; N, 10.30. Found: C, 66.22; H, 3.94; N, 10.30.

4.2.8.2. N–(3–(3–(4–Bromophenyl)–1–(4–chlorophenyl)–4,5–dihydro–1H–pyrazol–5–yl)phenyl)–7–chloroquinolin–4–amine ***12b***. Yellow solid; 90% yield; m.p. 274–276 °C. FTIR (ATR) ν (cm^{-1}): 3341 (NH), 3021 (=C–H), 1602 and 1546 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 3.25 (dd, J = 17.7, 6.1 Hz, 1H, H_A), 4.00 (dd, J = 17.7, 12.4 Hz, 1H, H_M), 5.64 (dd, J = 12.4, 6.1 Hz, 1H, H_X), 6.73 (d, J = 6.6 Hz, 1H, H_3), 7.03 (d, J = 8.2 Hz, 2H, H_{Co}), 7.26 (d, J = 8.2 Hz, 2H, H_{Cm}), 7.33–7.41 (m, 2H, $H_{Ao'}$, H_{Ap}), 7.43 (d, J = 8.5 Hz, 1H, H_{Ao}), 7.55 (t, J = 7.8 Hz, 1H, H_{Am}), 7.68 (d, J = 8.6 Hz, 2H, H_{Bm}), 7.74 (d, J = 8.6 Hz, 2H, H_{Bo}), 7.85 (dd, J = 9.0, 1.9 Hz, 1H, H_6), 8.19 (d, J = 1.9 Hz, 1H, H_8), 8.43–8.47 (m, 2H, H_2, H_5), 11.09 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 42.0, 58.4, 101.5, 114.5, 117.8, 120.0, 121.8, 122.0, 124.1, 124.6, 125.6, 126.6, 127.3, 128.6, 129.6, 130.6, 131.4, 132.6, 134.5, 139.9, 142.3, 147.6, 148.8, 150.3, 155.6, 159.4. MS (70 eV) m/z (%): 586/588 [M^+] (60/100), 476 (46), 447 (28), 254 (43), 91 (10). Anal. Calcd. For $C_{30}H_{21}BrCl_2N_4$: C, 61.25; H, 3.60; N, 9.52. Found: C, 61.23; H, 3.56; N, 9.50.

4.2.8.3. 7–Chloro–N–(3–(1–(4–chlorophenyl)–3–(4–methoxyphenyl)–4,5–dihydro–1H–pyrazol–5–yl)phenyl) quinolin–4–amine ***12c***. Yellow solid; 80% yield; m.p. 271–273 °C. FTIR (ATR) ν (cm^{-1}): 3345 (NH), 3020 (=C–H), 1607 and 1546 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 3.22 (dd, J = 17.6, 5.9 Hz, 1H, H_A), 3.82 (s, 3H, OCH_3), 3.98 (dd, J = 17.6, 12.2 Hz, 1H, H_M), 5.63 (dd, J = 12.2, 5.9 Hz, 1H, H_X), 6.73 (d, J = 6.1 Hz, 1H, H_3), 6.98 (d, J = 8.9 Hz, 2H, H_{Bm}), 7.04 (d, J = 8.7 Hz, 2H, H_{Co}), 7.26 (d, J = 8.7 Hz, 2H, H_{Cm}), 7.32–7.35 (m, 2H, $H_{Ao'}$, H_{Ap}), 7.40–7.47 (m, 2H, H_{Ao}, H_{Am}), 7.73 (d, J = 8.9 Hz, 2H, H_{Bo}), 7.90 (dd, J = 9.1, 2.1 Hz, 1H, H_6), 8.17 (d, J = 2.1 Hz, 1H, H_8), 8.47 (d, J = 6.1 Hz, 1H, H_2), 8.54 (d, J = 9.1 Hz, 1H, H_5), 11.08 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 41.9, 56.1, 58.5, 101.2, 114.1, 114.3, 117.1, 120.4, 122.2, 122.9, 123.0, 124.3, 125.1, 126.5, 126.6, 128.3, 130.1, 132.4, 135.9, 139.2, 142.9, 148.4, 150.6, 156.0, 159.7, 160.5, 163.0. MS (70 eV) m/z (%): 538/540 [M^+] (100/63), 428 (69), 399 (32), 254 (23), 91 (10). Anal. Calcd. For $C_{31}H_{24}Cl_2N_4O$: C, 69.02; H, 4.48; N, 10.39. Found: C, 69.00; H, 4.51; N, 10.36.

4.2.8.4. 7–Chloro–N–(3–(1–(4–chlorophenyl)–3–(3,4,5–trimethoxyphenyl)–4,5–dihydro–1H–pyrazol–5–yl) phenyl)quinolin–4–amine ***12d***. Yellow solid; 71% yield; m.p. 242–244 °C. FTIR (ATR) ν (cm^{-1}): 3377 (NH), 3030 (=C–H), 1609 and 1550 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 3.32 (dd, J = 17.7, 5.8 Hz, 1H, H_A), 3.70 (s, 3H, OCH_3), 3.84 (s, 6H, OCH_3), 3.98 (dd, J = 17.7, 12.3 Hz, 1H, H_M), 5.65 (dd, J = 12.3, 5.8 Hz, 1H, H_X), 6.72 (d, J = 6.8 Hz, 1H, H_3), 7.04–7.07 (m, 4H, H_{Bo}, H_{Co}), 7.13 (d, J = 7.7 Hz, 1H, H_{Ap}), 7.15–7.24 (m, 3H, $H_{Ao'}$, H_{Cm}), 7.36 (d, J = 8.0 Hz, 1H, H_{Ao}), 7.50 (t, J = 7.8 Hz, 1H, H_{Am}), 7.83 (dd, J = 9.0, 2.1 Hz, 1H, H_6), 8.17 (d, J = 2.1 Hz, 1H, H_8), 8.47–8.56 (m, 2H, H_2, H_5), 11.07 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 42.6, 56.1, 58.2, 60.1, 101.7, 103.9, 114.6, 117.4, 119.9, 121.9, 122.0, 125.0, 125.2, 126.1, 127.0, 129.5, 132.9, 135.1, 135.7, 139.3, 140.4, 143.3, 150.0, 150.1, 152.7, 156.4, 159.8, 163.1. MS (70 eV) m/z (%): 598/600 [M^+] (100/71), 488 (97), 459 (34), 254 (10), 91 (9). Anal. Calcd. For $C_{33}H_{28}Cl_2N_4O_3$: C, 66.11; H, 4.71; N, 9.35. Found: C, 66.09; H, 4.67; N, 9.35.

4.2.8.5. 7–Chloro–N–(3–(1–(4–chlorophenyl)–3–(p–tolyl)–4,5–dihydro–1H–pyrazol–5–yl)phenyl)quinolin–4–amine ***12e***. Yellow solid; 77% yield; m.p. 254–256 °C. FTIR (ATR) ν (cm^{-1}): 3331 (NH), 3022 (=C–H), 1604 and 1545 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 2.36 (s, 3H, CH_3), 3.21 (dd, J = 17.6, 6.0 Hz, 1H, H_A), 3.98 (dd, J = 17.6, 12.3 Hz, 1H, H_M), 5.63 (dd, J = 12.3, 6.0 Hz, 1H, H_X), 6.76 (d, J = 6.8 Hz, 1H, H_3), 7.07 (d, J = 8.2 Hz, 2H, H_{Co}), 7.09 (d, J = 7.7 Hz, 1H, H_{Ap}), 7.15–7.26 (m, 5H, $H_{Ao'}$, H_{Bm}, H_{Cm}), 7.32 (d, J = 8.0 Hz, 1H, H_{Ao}), 7.48 (t, J = 7.8 Hz, 1H, H_{Am}), 7.76–7.81 (m, 3H, H_6, H_{Bo}), 8.15 (d, J = 2.0 Hz, 1H, H_8), 8.46 (d, J = 6.8 Hz, 1H, H_2), 8.85 (d, J = 9.1 Hz, 1H, H_5), 11.05 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 21.2, 42.3, 57.9, 102.0, 114.7, 117.5, 120.5, 121.8, 122.4, 124.7, 125.2, 125.6, 126.6, 127.3, 127.7, 129.3, 129.9, 132.8, 135.2, 139.4, 140.3, 142.9, 146.6, 149.5, 149.6, 155.8, 159.2. MS (70 eV) m/z (%): 522/524 [M^+] (10/6), 412 (23), 383 (49), 254 (22), 91 (100). Anal. Calcd. For $C_{31}H_{24}Cl_2N_4$: C, 71.13; H, 4.62; N, 10.70. Found: C, 71.15; H, 4.59; N, 10.67.

4.2.8.6. 7–Chloro–N–(3–(1–(4–chlorophenyl)–3–phenyl–4,5–dihydro–1H–pyrazol–5–yl)phenyl)quinolin–4–amine ***12f***. Yellow solid; 72% yield; m.p. 263–265 °C. FTIR (ATR) ν (cm^{-1}): 3333 (NH), 3020 (=C–H), 1606 and 1538 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 3.25 (dd, J = 17.7, 6.0 Hz, 1H, H_A), 4.01 (dd, J = 17.7, 12.3 Hz, 1H, H_M), 5.64 (dd, J = 12.3, 6.0 Hz, 1H, H_X), 6.73 (d, J = 5.7 Hz, 1H, H_3), 7.04 (d, J = 8.2 Hz, 2H, H_{Co}), 7.11 (d, J = 7.7 Hz, 1H, H_{Ap}), 7.17–7.24 (m, 3H, $H_{Ao'}$, H_{Cm}), 7.33 (d, J = 8.0 Hz, 1H, H_{Ao}), 7.42–7.45 (m, 4H, H_{Am}, H_{Bm}, H_{Bp}), 7.75–7.85 (m, 3H, H_6, H_{Bo}), 8.15 (d, J = 2.1 Hz, 1H, H_8), 8.43–8.48 (m, 2H, H_2, H_5), 11.07 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 42.5, 58.8, 101.0, 114.4, 117.8, 119.7, 121.7, 121.8, 124.9, 125.3, 126.0, 126.3, 127.7, 128.9, 130.3, 130.7, 130.8, 132.4, 135.0, 139.7, 142.8, 147.1, 149.0, 149.7, 156.7, 159.1. MS (70 eV) m/z (%): 508/510 [M^+] (25/16), 398 (59), 369 (100), 254 (39), 91 (22). Anal. Calcd. For $C_{30}H_{22}Cl_2N_4$: C, 70.73; H, 4.35; N, 11.00. Found: C, 70.77; H, 4.33; N, 10.99.

4.2.9. General procedure for the synthesis of N–(3–(3–aryl–1–(3,5–dichlorophenyl)–4,5–dihydro–1H–pyrazol–5–yl)phenyl)–7–chloroquinolin–4–amines 13a–f

A mixture of the chalcone **8a**–**f** (1.00 mmol) and 3,5–dichlorophenylhydrazine hydrochloride (2.00 mmol) in methanol (10.0 mL) was heated under reflux for 3 h. After cooling, the solid formed was filtered and washed with water and methanol.

4.2.9.1. 7–Chloro–N–(3–(3–(4–chlorophenyl)–1–(3,5–dichlorophenyl)–4,5–dihydro–1H–pyrazol–5–yl)phenyl) quinolin–4–amine ***13a***. Pale yellow solid; 92% yield; m.p. 239–241 °C. FTIR (ATR) ν (cm^{-1}): 3343 (NH), 3024 (=C–H), 1598 and 1542 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 3.32 (dd, J = 17.9, 5.4 Hz, 1H, H_A), 4.03 (dd, J = 17.9, 12.2 Hz, 1H, H_M), 5.75 (dd, J = 12.2, 5.4 Hz, 1H, H_X), 6.73 (d, J = 6.8 Hz, 1H, H_3), 6.90 (d, J = 1.5 Hz, 1H, H_{Cp}), 7.01 (d, J = 1.5 Hz, 2H, H_{Co}), 7.33–7.40 (m, 2H, $H_{Ao'}$, H_{Ap}), 7.43 (d, J = 8.0 Hz, 1H, H_{Ao}), 7.52 (d, J = 8.6 Hz, 2H, H_{Bm}),

7.59 (t, J = 8.0 Hz, 1H, H_{Am}), 7.78–7.88 (m, 3H, H_6, H_{Bo}), 8.14 (d, J = 1.8 Hz, 1H, H_8), 8.44 (d, J = 6.8 Hz, 1H, H_2), 8.74 (d, J = 9.1 Hz, 1H, H_5), 11.05 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 42.3, 58.9, 102.0, 114.7, 117.9, 119.4, 121.0, 121.8, 124.6, 125.3, 127.0, 127.4, 129.0, 129.1, 129.8, 130.1, 132.7, 133.7, 135.1, 140.2, 142.9, 148.4, 148.9, 151.4, 155.3, 159.6. MS (70 eV) *m/z* (%): 576/578 [M^+] (64/82), 466 (68), 437 (58), 254 (100), 91 (30). Anal. Calcd. For $C_{30}H_{20}Cl_4N_4$: C, 62.31; H, 3.49; N, 9.69. Found: C, 62.34; H, 3.46; N, 9.69.

4.2.9.2. N–(3–(3–(4–Bromophenyl)–1–(3,5–dichlorophenyl)–4,5–dihydro–1H–pyrazol–5–yl)phenyl)–7–chloroquinolin–4–amine ***13b***. Pale yellow solid; 89% yield; m.p. > 300 °C. FTIR (ATR) ν (cm^{-1}): 3339 (NH), 3022 (=C–H), 1600 and 1547 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 3.32 (dd, J = 17.9, 5.4 Hz, 1H, H_A), 4.04 (dd, J = 17.9, 12.3 Hz, 1H, H_M), 5.75 (dd, J = 12.3, 5.4 Hz, 1H, H_X), 6.71 (d, J = 6.6 Hz, 1H, H_3), 6.76 (d, J = 1.5 Hz, 1H, H_{Cp}), 7.02 (d, J = 1.5 Hz, 2H, H_{Co}), 7.05 (d, J = 7.6 Hz, 1H, H_{Ap}), 7.26–7.32 (m, 2H, $H_{Ao'}$, H_{Ao}), 7.43 (t, J = 7.8 Hz, 1H, H_{Am}), 7.61 (dd, J = 9.0, 1.9 Hz, 1H, H_6), 7.72–7.75 (m, 4H, H_{Bo}, H_{Bm}), 8.17 (d, J = 1.9 Hz, 1H, H_8), 8.45–8.47 (m, 2H, H_2, H_5), 11.05 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 42.0, 58.9, 101.6, 114.5, 117.5, 120.1, 121.9, 122.1, 124.2, 124.6, 125.0, 126.4, 127.3, 128.2, 130.0, 130.4, 131.4, 132.6, 134.7, 140.2, 142.6, 147.1, 148.7, 151.0, 155.2, 159.3. MS (70 eV) *m/z* (%): 620/622 [M^+] (52/100), 510 (89), 481 (57), 254 (14), 91 (27). Anal. Calcd. For $C_{30}H_{20}BrCl_3N_4$: C, 57.86; H, 3.24; N, 9.00. Found: C, 57.87; H, 3.25; N, 8.96.

4.2.9.3. 7–Chloro–N–(3–(1–(3,5–dichlorophenyl)–3–(4–methoxyphenyl)–4,5–dihydro–1H–pyrazol–5–yl)phenyl) quinolin–4–amine ***13c***. Pale yellow solid; 75% yield; m.p. > 300 °C. FTIR (ATR) ν (cm^{-1}): 3343 (NH), 3021 (=C–H), 1605 and 1547 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 3.29 (dd, J = 17.8, 5.2 Hz, 1H, H_A), 3.81 (s, 3H, OCH_3), 4.02 (dd, J = 17.8, 12.1 Hz, 1H, H_M), 5.74 (dd, J = 12.1, 5.2 Hz, 1H, H_X), 6.74 (d, J = 6.1 Hz, 1H, H_3), 6.80 (d, J = 1.5 Hz, 1H, H_{Cp}), 7.00–7.02 (m, 4H, H_{Bm}, H_{Co}), 7.17 (d, J = 7.7 Hz, 1H, H_{Ap}), 7.30–7.37 (m, 3H, $H_{Ao'}$, H_{Ao}, H_{Am}), 7.64 (dd, J = 9.1, 2.1 Hz, 1H, H_6), 7.74 (d, J = 8.9 Hz, 2H, H_{Bo}), 8.15 (d, J = 2.1 Hz, 1H, H_8), 8.46 (d, J = 6.1 Hz, 1H, H_2), 8.53 (d, J = 9.1 Hz, 1H, H_5), 11.02 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 42.1, 55.9, 58.0, 100.6, 114.3, 114.6, 117.7, 120.5, 122.3, 122.7, 123.6, 124.2, 125.2, 126.5, 126.6, 128.2, 130.4, 132.7, 135.7, 139.2, 143.1, 148.5, 150.7, 155.9, 159.7, 161.0, 163.0. MS (70 eV) *m/z* (%): 572/574 [M^+] (100/92), 462 (29), 433 (72), 254 (63), 91 (48). Anal. Calcd. For $C_{31}H_{23}Cl_3N_4O$: C, 64.88; H, 4.04; N, 9.76. Found: C, 64.95; H, 3.99; N, 9.82.

4.2.9.4. 7–Chloro–N–(3–(1–(3,5–dichlorophenyl)–3–(3,4,5–trimethoxyphenyl)–4,5–dihydro–1H–pyrazol–5–yl) phenyl)quinolin–4–amine ***13d***. Pale yellow solid; 73% yield; m.p. 203–205 °C. FTIR (ATR) ν (cm^{-1}): 3374 (NH), 3032 (=C–H), 1607 and 1551 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 3.39 (dd, J = 17.9, 5.1 Hz, 1H, H_A), 3.70 (s, 3H, OCH_3), 3.82 (s, 6H, OCH_3), 4.02 (dd, J = 17.9, 12.2 Hz, 1H, H_M), 5.76 (dd, J = 12.2, 5.1 Hz, 1H, H_X), 6.75 (d, J = 6.8 Hz, 1H, H_3), 6.79 (d, J = 1.5 Hz, 1H, H_{Cp}), 7.04–7.06 (m, 4H, H_{Bo}, H_{Co}), 7.13 (d, J = 7.7 Hz, 1H, H_{Ap}), 7.24–7.34 (m, 2H, $H_{Ao'}$, H_{Ao}), 7.54 (t, J = 7.8 Hz, 1H, H_{Am}), 7.61 (dd, J = 9.0, 2.1 Hz, 1H, H_6), 8.17 (d, J = 2.1 Hz, 1H, H_8), 8.47–8.54 (m, 2H, H_2, H_5), 11.08 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 42.3, 56.1, 58.2, 60.7, 102.1, 104.4, 114.6, 117.5, 119.7, 122.1, 122.3, 124.9, 125.3, 126.4, 127.1, 129.9, 132.9, 135.1, 135.7, 139.5, 140.2, 143.0, 149.5, 150.0, 152.7, 156.5, 159.2, 163.2. MS (70 eV) *m/z* (%): 632/634 [M^+] (100/93), 522 (13), 493 (6), 254 (15), 91 (40). Anal. Calcd. For $C_{33}H_{27}Cl_3N_4O_3$: C, 62.52; H, 4.29; N, 8.84. Found: C, 62.50; H, 4.31; N, 8.84.

4.2.9.5. 7–Chloro–N–(3–(1–(3,5–dichlorophenyl)–3–(p–tolyl)–4,5–dihydro–1H–pyrazol–5–yl)phenyl)quinolin–4–amine ***13e***. Pale yellow solid; 80% yield; m.p. 270–272 °C. FTIR (ATR) ν (cm^{-1}): 3329 (NH), 3024 (=C–H), 1602 and 1546 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 2.37 (s, 3H, CH_3), 3.28 (dd, J = 17.8, 5.3 Hz, 1H, H_A), 4.02 (dd, J = 17.8, 12.2 Hz, 1H, H_M), 5.74 (dd, J = 12.2, 5.3 Hz, 1H, H_X), 6.72 (d, J = 6.8 Hz, 1H, H_3), 6.82 (d, J = 1.5 Hz, 1H, H_{Cp}), 7.04 (d, J = 1.5 Hz, 2H, H_{Co}), 7.08 (d, J = 7.7 Hz, 1H, H_{Ap}), 7.15–7.27 (m, 3H, $H_{Ao'}$, H_{Bm}), 7.33 (d, J = 8.0 Hz, 1H, H_{Ao}), 7.44 (t, J = 7.8 Hz, 1H, H_{Am}), 7.66–7.71 (m, 3H, H_6, H_{Bo}), 8.16 (d, J = 2.0 Hz, 1H, H_8), 8.46 (d, J = 6.8 Hz, 1H, H_2), 8.53 (d, J = 9.1 Hz, 1H, H_5), 11.04 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 20.5, 42.5, 58.2, 102.0, 114.5, 117.9, 120.3, 121.8, 122.6, 124.6, 125.0, 125.6, 126.5, 127.2, 127.6, 129.3, 129.8, 132.6, 135.2, 139.6, 140.2, 142.9, 146.6, 149.7, 149.9, 155.9, 159.7. MS (70 eV) *m/z* (%): 556/558 [M^+] (29/28), 446 (62), 417 (20), 254 (100), 91 (64). Anal. Calcd. For $C_{31}H_{23}Cl_3N_4$: C, 66.74; H, 4.16; N, 10.04. Found: C, 66.76; H, 4.20; N, 9.97.

4.2.9.6. 7–Chloro–N–(3–(1–(3,5–dichlorophenyl)–3–phenyl–4,5–dihydro–1H–pyrazol–5–yl)phenyl)quinolin–4–amine ***13f***. Pale yellow solid; 75% yield; m.p. 199–201 °C. FTIR (ATR) ν (cm^{-1}): 3331 (NH), 3021 (=C–H), 1604 and 1539 (C=N and C=C). ^{1}H NMR (400 MHz, DMSO–d_6) δ ppm 3.32 (dd, J = 17.9, 5.3 Hz, 1H, H_A), 4.05 (dd, J = 17.9, 12.2 Hz, 1H, H_M), 5.75 (dd, J = 12.2, 5.7 Hz, 1H, H_X), 6.73 (d, J = 6.3 Hz, 1H, H_3), 6.83 (d, J = 1.5 Hz, 1H, H_{Cp}), 7.07 (d, J = 1.5 Hz, 2H, H_{Co}), 7.15 (d, J = 7.7 Hz, 1H, H_{Ap}), 7.26–7.35 (m, 2H, $H_{Ao'}$, H_{Ao}), 7.48–7.50 (m, 4H, H_{Am}, H_{Bm}, H_{Bp}), 7.62 (dd, J = 9.1, 2.1 Hz, 1H, H_6), 7.74–7.80 (m, 2H, H_{Bo}), 8.15 (d, J = 2.1 Hz, 1H, H_8), 8.46–8.51 (m, 2H, H_2, H_5), 11.07 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO–d_6) δ ppm 42.5, 58.6, 101.0, 114.4, 117.8, 119.8, 121.9, 122.0, 125.0, 125.1, 126.5, 126.5, 127.7, 128.5, 129.9, 130.6, 131.1, 132.3, 135.0, 140.0, 142.4, 147.7, 149.6, 149.9, 155.0, 159.2. MS (70 eV) *m/z* (%): 542/544 [M^+] (55/54), 432 (62), 403 (20), 254 (67), 91 (100). Anal. Calcd. For $C_{30}H_{21}Cl_3N_4$: C, 66.25; H, 3.89; N, 10.30. Found: C, 66.25; H, 3.92; N, 10.29.

4.3. Anticancer activity

The human cancer cell lines of the cancer screening panel were grown in an RPMI–1640 medium containing 5% fetal bovine serum and 2 mM L–glutamine. For a typical screening experiment, cells were inoculated into 96–well microtiter plates. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to the addition of the tested compounds. After 24 h, two plates of each cell line were fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of sample addition (Tz). The samples were solubilized in dimethyl sulfoxide (DMSO) at 400–fold the desired final maximum test concentration and stored frozen prior to use. At the time of compound addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/mL gentamicin. An additional four 10–fold or 1/2 log serial dilutions were made to provide a total of five drug concentrations plus the control. Aliquots of 100 μL of these different sample dilutions were added to the appropriate microtiter wells already containing 100 μL of medium, resulting in the required final sample concentrations [53]. After the tested compounds were added, the plates were incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay was

terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 μL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded, and plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. Using the seven absorbance measurements [time zero (Tz), control growth in the absence of drug (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as: $[(Ti - TZ)/(C - TZ)] \times 100$ for concentrations for which $Ti > Tz$, and $[(Ti - TZ)/TZ] \times 100$ for concentrations for which $Ti < Tz$. Two dose–response parameters were calculated for each compound. Growth inhibition of 50% (GI_{50}) was calculated from $[(Ti - TZ)/(C - TZ)] \times 100 = 50$, which is the drug concentration resulting in a 50% lower net protein increase in the treated cells (measured by SRB staining) as compared to the net protein increase seen in the control cells and the LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning), indicating a net loss of cells; calculated from $[(Ti - TZ)/TZ] \times 100 = -50$. Values were calculated for each of these two parameters if the level of activity is reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested [53–55].

4.4. Antifungal activity

4.4.1. Microorganisms and media

For the antifungal evaluation, standardized strains from the American Type Culture Collection (ATCC), Rockville, MD, USA, *C. albicans* ATCC 10231 and *C. neoformans* ATCC 32264 were used. Strains were grown on Sabouraud–chloramphenicol agar slants for 48 h at 30 °C, were maintained on slopes of Sabouraud–dextrose agar (SDA, Oxoid) and sub–cultured every 15 days to prevent pleomorphic transformations. Inocula were obtained according to reported procedures [51] and adjusted to $1–5 \times 10^3$ cells with colony forming units (CFU)/mL.

4.4.2. Fungal growth inhibition percentage determination

Broth microdilution techniques were performed in 96–well microplates according to the Clinical and Laboratory Standards Institute Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard M27–A3 [51]. For the assay, compound test wells (CTWs) were prepared with stock solutions of each compound in DMSO (maximum concentration $\leq$ 1%), diluted with RPMI–1640, to final concentrations of 250–0.98 μg/mL. An inoculum suspension (100 μL) was added to each well (final volume in the well = 200 μL). A growth control well (GCW) (containing medium, inoculum, and the same amount of DMSO used in a CTW, but compound–free) and a sterility control well (SCW) (sample, medium, and sterile water instead of inoculum) were included for each fungus tested. Microtiter trays were incubated in a moist, dark chamber at 30 °C for 48 h for both yeasts. Microplates were read in a VERSA Max microplate reader (Molecular Devices, Sunnyvale, CA, USA). Amphotericin B (SIGMA–ALDRICH, St Louis, MO, USA) was used as positive control. Tests were performed in triplicate. Reduction of growth for each compound concentration was calculated as follows: % of inhibition = 100 − (OD 405 CTW − OD 405 SCW)/(OD 405 GCW − OD 405 SCW). The means ± SEM were used for constructing the dose–response curves representing % inhibition *vs* concentration of each compound.

4.5. Antibacterial activity

All the synthesized compounds were tested to determine the antibacterial activity against gram–negative and gram–positive bacteria. Wild–type and multi–resistant strains were included as follows: methicillin–susceptible *Staphylococcus aureus* ATCC 25923 (MSSA), methicillin–resistant *Staphylococcus aureus* ATCC 43300 (MRSA), vancomycin–intermediate *Staphylococcus aureus* (VISA), *Escherichia coli* ATCC 25922, carbapenemase–positive *Klebsiella pneumoniae* BAA 1705, *Klebsiella pneumoniae* ATCC 700603 (extended spectrum beta lactamase, ESBL positive), *Pseudomonas aeruginosa* ATCC 27853 and *Neisseria gonorrhoeae* ATCC 31426 (beta lactamase positive). Stock solutions (100 mg/mL) of the compounds were prepared in dimethyl sulfoxide (DMSO) and diluted to a final screening concentration of 1 mg/mL. An initial screening of bacterial inhibition was performed by agar diffusion method. Briefly, sterile Mueller Hinton agar (MHA, BBL) was prepared in Petri dishes and inoculated with a bacterial suspension prepared in trypticase soy broth and adjusted to 1.5×10^8 colony forming unit CFU/mL (i.e. 0.08–0.1 OD at 600 nm) [56]. Wells (6 mm in diameter) were punched in the agar and 10 μL of each compound (stock solution) were filled into each well. Dimethyl sulfoxide and trypticase soy broth were included as negative controls (i.e. no inhibition of bacterial growth). Gentamicin and polymyxin B (Sigma, Aldrich) were included as positive controls of growth inhibition. Compounds showing growth inhibition were tested at least twice before being selected for microdilution testing. For *N. gonorrhoeae* the agar diffusion method was also used in the screening process with some modifications. For this method, 200 μL of a bacterial suspension (1.5×10^8 CFU/mL) was inoculated in gonococcal (GC) agar (BBL) supplemented with 1% isovitalex (BBL), then the compounds were added to the wells as above mentioned and incubated at 35–36.5 °C in 5% CO_2 atmosphere for 48 h. Penicillin, ceftriaxone and ciprofloxacin (BBL) were used as controls [57].

4.5.1. Microdilution test

Minimum inhibitory concentration (MIC) was determined in those compounds with visible and reproducible antibacterial inhibition by the screening test. Bacterial suspensions were adjusted with Mueller Hinton broth (MHB) to a concentration of $5 \times 10^5–8 \times 10^5$ [56]. Stock solutions of the newly synthesized compounds were diluted in MHB containing 5% DMSO and 0.1% Tween 80 [58] and added to 90 μL of the bacterial inoculum. The microplates were incubated for 24 h at 35–37 °C. MICs were defined the lowest concentration with visible inhibition of bacterial growth [56] and/or detection using resazurin (1 mg/mL). Minimum bactericidal concentrations (MBC) were determined by culturing 3 μL of each dilution with no visible growth into trypticase agar plates. Gentamicin (Sigma, Aldrich) was included as control of inhibition growth; MHB and DMSO were used as a negative control. Experiments were performed in duplicate and replicated at least three times.

For *N. gonorrhoeae*, those compounds that produced visible growth inhibition in the screening test were further tested for MIC on agar plates as described by the Centers for Disease Control and Prevention [59] and the Clinical and Laboratory Standards Institute

[57] with modifications. Briefly GC agar supplemented with 1% isovitalex was prepared containing increasing concentrations of the novel compounds and inoculated with 5 μL of the bacterial suspension (i.e. 1×10^4 CFU) [59]. The lowest concentration of the compound that inhibited bacterial growth was determined as the MIC. Bacterial growth was examined and verified using the oxidase test. Experiments were performed in duplicate and replicated at least three times.

4.6. Antiprotozoal activity

4.6.1. Compounds storage

All compounds were stored at room temperature until use. Prior to the biological evaluation, each compound was solubilized in 0.5% DMSO and then, diluted to the appropriate concentration in culture media.

4.6.2. Antiplasmodial activity

The antiplasmodial activity was evaluated *in vitro* on asynchronic cultures of *P. falciparum* (3D7 strain), maintained in standard culture conditions. The effect of each compound over the growth of the parasites was determined by quantification of parasite death, based on the measurement of lactate dehydrogenase (LDH) activity released from the cytosol of damaged cells into the supernatant. Briefly, unsynchronized *P. falciparum* cultures were adjusted to 0.5% parasitemia and 1% hematocrit in RPMI medium enriched with 3% Lipid–rich bovine serum albumin – Albumax II. Then, in each well of a 96–wells plate 100 μL of parasite suspension were dispensed and subsequently exposed against 100 μL of four serial dilutions of compounds (100, 25, 6.25 and 1.56 μg/mL). Dilutions were prepared from a stock solution of 1000 μg/mL. Chloroquine (CQ) was used as positive antiplasmodial drug control. Parasites unexposed to any compound were used as control of both growth and viability (negative control). Plates were incubated for 48 h at 37 °C in N_2 (90%), CO_2 (5%) and O_2 (5%) atmosphere. After incubation, plates were harvested and parasites were subjected to three 20-min freeze–thaw cycles. Meanwhile, 100 μL of Malstat reagent (400 μL Triton X–100 in 80 mL deionized water, 4 g L–lactate, 1.32 g Tris buffer and 0.022 g acetylpyridine adenine dinucleotide in 200 mL deionized water; pH 9.0) and 25 μL of NBT/PES solution (0.16 g nitroblue tetrazolium salt and 0.08 g phenazine ethosulphate in 100 mL deionizated water) were added to each well of a second flat–bottomed 96–well microtiter plate. After freeze–thaw cycles, culture in each of the wells of the first plate was resuspended by pipetting and 15 μL of each well was taken and added to the corresponding well of the second plate (containing Malstat and NBT/PES reagents). After an hour of incubation in the dark, color development of the LDH reaction was monitored colorimetrically in a spectrofluorometer (Varioskan, Thermo) reading at 650 nm. The intensity of color in each experimental condition was registered as Optical Densities (O.D). Non–specific absorbance was corrected by subtracting O.D of the blank. Determinations were done by triplicate in at least two independent experiments [60].

4.6.3. Hemolytic activity

The ability to induce hemolysis was evaluated specifically to compounds which showed antiplasmodial activity following the method of cytotoxicity by spectrophotometry on 96–well plates. Human Red blood cells (huRBC), adjusted to 5% hematocrit in RPMI–1640 medium. Five hundred μL were placed into each well of 24–well plate and subsequently exposed against 200 μg/mL. Detection of free hemoglobin, after 48 h of incubation at 37 °C, is the evidence that the compound induces hemolysis. The concentration of free hemoglobin was performed spectrophotometrically at 542 nm (Varioskan, Thermo) and intensity of color (absorbance) was registered as optical densities (O.D). Non–specific absorbance was corrected by subtracting absorbance of the blank. Determinations were done by triplicate in at least two independent experiments [61].

4.6.4. Cytotoxic activity

Cytotoxicity of the compounds was evaluated over human monocytes (U–937 ATCC CRL–1593.2) in exponential growing phase and adjusted at 1×10^5 cells/mL in RPMI–1640 enriched with 10% fetal bovine serum (FBS). One hundred μL of cell suspension were dispensed in each well of a 96–wells microplate and then, 100 μL of 200–50–12.5 and 3.125 μg/mL concentration of each compound or standard drugs chloroquine (malaria), benznidazole (chagas) and amphotericin B (leishmaniasis) was added. Cell exposed to compounds or standard drugs were incubated 72 h at 37 °C and 5% of CO_2. Cytotoxic activity of each compound was determined according to the effect on the cell viability by the MTT microenzimatic method in which 3–(4,5–dimethylthiazol–2–yl)–2,5–diphenyltetrazolium bromide is reduced to a purple product named formazan by mitochondrial enzyme succinate dehydrogenase. Thus, 10 μL/well were added to each well of exposed and unexposed cells, and plates were incubated at 37 °C, 5% CO_2 during 3 h. The reaction was stopped by adding 100 μL/well of isopropanol with 50% and 10% of SDS (sodium dodecyl sulfate). The concentration of formazan was determined spectrophotometrically at 570 nm (Varioskan, Thermo) and intensity of color (absorbance) was registered as O.D. Cells exposed to control drugs (chloroquine, amphotericin B and benznidazole) were used as control for toxicity (positive control) while cell incubated in absence of any compound or drug were used as control for viability (negative control). Non–specific absorbance was corrected by subtracting absorbance (O.D) of the blank. Determinations were done by triplicate in at least two independent experiments [62].

4.6.5. Antitrypanosomal activity

The effectiveness of synthesized compounds was determined according to the ability of the compound to reduce the infection by *T. cruzi* parasites. For this, the antitrypanosomal activity was tested on intracellular amastigotes of *T. cruzi* (Tulahuen strain transfected with β–galactosidase gene, donated by Dr. F. S. Buckner, University of Washington) [63]. For this purpose, 100 μL of U–937 human cells at a concentration of 2.5×10^5 cells/mL in RPMI–1640, 10% SFB and 0.1 μg/mL of PMA were placed in each well of 96–wells microplate. Cells were then infected with epimastigotes (24 h of growing) in 5:1 parasites per cell relation. Infected cells were incubated with each compound at four concentrations (50, 12.5, 3.125 and 0.78 μg/mL) using benznidazole as trypanocidal control. After 72 h of incubation, the effect of all compounds on intracellular amastigotes viability was determined measuring the β–galactosidase activity by colorimetric method. For this assay, CPRG at 100 μM and nonidet P–40 at 0.1% were added, and after 3 h of incubation, measurement was performed at 570 nm in a spectrophotometer (Varioskan, Thermo) and intensity of color (absorbance) was registered as optical densities (O.D). Infected cells exposed to control drugs (benznidazole) were used as a control for antitrypanosomal activity (positive control) while infected cells incubated in absence of any compound or drug were used as control for infection (negative control). Non–specific absorbance was corrected by subtracting

absorbance (O.D) of the blank. Determinations were done by triplicate in at least two independent experiments [64].

4.6.6. Antileishmanial activity

Antileishmanial activity of compounds was determined according to the ability of the compound to reduce the infection by *L. panamensis* parasites. For this, the antileishmanial activity was tested on intracellular amastigotes of *L. panamensis* transfected with the green fluorescent protein gene (MHOM/CO/87/UA140–EpiR–GFP strain) [65]. Briefly, U–937 human cells at a density of 3×10^5 cells/mL in RPMI 1640 and 0.1 μg/mL of PMA (phorbol–12–myristate–13–acetate) were dispensed on 24–wells microplate and then infected with stationary phase growing *L. panamensis* promastigotes in a 15:1 parasites per cell ratio. Plates were incubated at 34 °C and 5% CO_2 for 3 h and then cells were washed twice with phosphate buffer solution (PBS) to eliminate not internalized parasites. Fresh RPMI–1640 was added into each well (1 mL) and plates were incubated again to complete infection. After 24 h of infection, the RPMI–1640 medium was replaced by fresh culture medium containing each compound at four serial dilutions (50, 12.5, 3.125 and 0.78 μg/mL) and plates were then incubated at 37 °C and 5% CO_2 during 72 h, then, cells were removed from the bottom plate with a trypsin/EDTA (250 mg) solution. The cells were centrifuged at 1100 rpm during 10 min at 4 °C, the supernatant was discarded and cells were washed with 1 mL of cold PBS and centrifuged at 1100 rpm for 10 min at 4 °C. Cells were washed two times employing PBS, as previously, and after the last wash, the supernatant was discarded and cells were suspended in 500 μL of PBS.

Cells were analyzed by flow cytometry employing a flow cytometer (cytomics FC 500MPL) reading at 488 nm (exciting) and 525 nm (emitting) over an argon laser and counting 10.000 events. Infected cells were determined according the events for green fluorescence (parasites). All determinations for each compound and standard drugs were carried out by triplicate, in two experiments. Infected cells exposed to control drugs (amphotericin B) were used as control for antileishmanial activity (positive control) while infected cells incubated in absence of any compound or drug were used as control for infection (negative control). Nonspecific fluorescence was corrected by subtracting fluorescence of unstained cells. Determinations were done by triplicate in at least two independent experiments [62,65].

4.6.7. Statistical analysis

Cytotoxicity was determined according to viability and mortality percentages obtained for each experimental condition (synthetized compounds, amphotericin B, benznidazole, chloroquine and culture medium). Results were expressed as mean lethal concentrations (LC_{50}), concentration necessary to kill 50% of cells, calculated by Probit analysis (Parametric method of linear regression that permits doses–response analysis) [66].

Initially, viability percentages were calculated by Equation (1), where the D.O of control well, corresponds to 100% of viability. In turn, mortality percentage corresponds to 100% − % viability.

$$\%\text{Viability} = \frac{\text{D.O Exposed cells}}{\text{D.O Control cells}} \times 100 \quad (1)$$

Then, the percentage of cell growth inhibition was calculated using equation (2):

$$\%\ \text{cell growth inhibition} = 100\%\ \text{viability} \quad (2)$$

The toxicity was defined according to LC_{50} values, using the follow scale: Toxic; $LC_{50} < 100$ μM; moderately toxic; $LC_{50} > 100$ μg/mL and <200 μM and potentially nontoxic; $LC_{50} > 200$ μM.

The anti–*Plasmodium* activity of each evaluated compound was evidenced by the reduction of the absorbance (O.D). Indeed, the viability percentage was calculated by Equation (3):

$$\text{Viability}(\%) = \frac{(\text{O.D})\ \text{of parasites exposed to compounds} - (\text{O.D})\ \text{of culture medium}}{(\text{O.D})\ \text{of parasites unexposed to compounds} - (\text{O.D})\ \text{of culture medium}} \times 100 \quad (3)$$

Then, the inhibition growing percentage was calculated according to the following formula (4):

$$\text{Inhibition}(\%) = 100 - \text{Viability}(\%) \quad (4)$$

The inhibitory concentration (IC_{50}) i.e. the concentration of the compound which produces a 50% reduction of growth of the parasite was determined by the statistical program Probit analysis.

Antileishmanial activity was determined according reduction of infected cells percentages obtained for each experimental condition by the cytometer. The parasitemia values for each concentration of compound tested were calculated by Equation (5), where the % of infected cells in the control well, corresponds to 100% of parasitemia.

$$\%\text{Infection} = \frac{\%\ \text{infected cells in compound exposed well}}{\%\ \text{infected cells in control well}} \times 100 \quad (5)$$

Then, inhibition percentage was calculated with formula (6):

$$\text{Inhibition}\ (\%) = 100 - \text{Parasitemia}\ (\%) \quad (6)$$

For trypanosomal activity, the parasitemia percentage and parasitemia inhibition were calculated by equation (5) and formula (6).

Results of antileishmanial and antitrypanosomal activities were expressed as half the maximal effective concentrations (EC_{50}) measured by Probit method. The activity of each compound was established according to EC_{50} values, using the follow scale: Active; $EC_{50} < 50$ μg/mL; moderately active; $EC_{50} > 50$ μg/mL and <100 μg/mL and potentially no active: $EC_{50} > 100$ μg/mL.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2017.03.016.

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