**Synthesis and *in Vitro* Antitumor Activity of a Novel Series of 2-Pyrazoline Derivatives Bearing the 4-Aryloxy-7-chloroquinoline Fragment**

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**Abstract:** A new series of NH-pyrazoline derivatives 6 was synthesized by cyclocondensation reaction of novel [(7-chloroquinolin-4-yl)oxy]chalcones 5 with hydrazine hydrate. The treatment of pyrazolines 6 with acetic anhydride or formic acid yielded the N-acetyl- or N-formylpyrazoline derivatives 7–8, respectively. These novel 2-pyrazoline derivatives 6–8 were evaluated by the U.S. National Cancer Institute (NCI). Compounds 7b,d,f and 8c,f showed remarkable antitumor activity against 58 cancer cell lines, with the most important GI50 values from *in vitro* assays ranging from 0.48 to 1.66 μM. The 2-pyrazoline derivatives bearing the 4-aryloxy-7-chloroquinoline fragment are thus considered to be useful leads for the rational design of new antitumor agents.

**Keywords:** microwave irradiation; Claisen-Schmidt condensation; chalcones; cyclocondensation reaction; 2-pyrazolines; antitumor activity
1. Introduction

The identification of novel structures that can be potentially useful in designing new, potent selective and less toxic anticancer agents is still a major challenge for medicinal chemistry researchers [1]. It is well known that many natural or synthetic chalcones are highly active in a large pharmaceutical and medicinal applications [2,3]. Several strategies for the synthesis of these systems based on formation of carbon-carbon new bonds have been reported and among them the direct Aldol and Claisen-Schmidt condensations still occupy prominent position [4]. Chalcones are found to be effective as antimicrobial [5], antiviral [6], cardiovascular [7] and anti-inflammatory [8] agents; as well as their recognized synthetic utility. After the pioneering works of Fischer and Knoevenagel in the late nineteenth century [9], the reaction of α,β-unsaturated aldehydes and ketones with hydrazines became one of the most popular method for the preparation of 2-pyrazolines, which have attracted interest due to their diverse biological activities such as antitumor, immunosuppressive, antibacterial, anti-inflammatory, anticancer, antidiabetic and antidepressants [1,10–16]. Among the existing various pyrazoline type derivatives, 1-acetylpyrazolines have been identified as one of the most promising scaffolds, which were found to display fungicidal and insecticidal activities [17]. Examples of such systems are shown in Figure 1.

**Figure 1.** Some pyrazolines with remarkable biological activity.

On the other hand, the quinoline motive occurs in several natural compounds (cinchona alkaloids) and pharmacologically active substances displaying a broad range of biological activity [18]. In recent years it have been reported that the incorporation of these active pharmacophores in the structure of new heterocyclic compounds could potentiate their biological activity [19,20]. Prompted by the above
mentioned biological properties of chalcones, pyrazolines and the additional value of having quinoline motives in their structures and in continuation with our current studies directed toward the synthesis of novel nitrogen containing heterocyclic compounds with biological activity [21–26], we have decided to explore a series of new pyrazolines containing the 4-aryloxy-7-chloroquinoline fragment in their structures derived from chalcones as starting materials. The results discussed in this paper reflect our efforts in discovering new potential anticancer chemotherapeutic agents.

2. Results and Discussion

2.1. Chemistry

In order to obtain the new key chalcone derivatives 5 as starting materials for the synthesis of the target products 6–8, the synthesis of the precursor 4-(7-chloroquinolin-4-yl)oxy)-3-methoxybenzaldehyde (3) was performed by the selective nucleophilic aromatic substitution (S_{N}Ar) of the 4-chlorine atom on 4,7-dichloroquinoline (1) with 4-hydroxy-3-methoxybenzaldehyde (2). This S_{N}Ar process was carried out by microwave irradiation of the reagents for 6 min at a power of 100 W and temperature of 100 °C. The present protocol is quite convenient and environmentally friendly, since the reaction proceeds under mild reaction conditions when compared to classical methods [27]. Then the Claisen-Schmidt condensation of precursor 3 with several aromatic acetophenones led to the formation of 5 in good to excellent yields (58%–95%) (Scheme 1 and Experimental Section).

**Scheme 1.** Synthesis of novel [(7-chloroquinolin-4-yl)oxy]chalcones 5.

\[ \begin{align*}
\text{Cl} & \quad \text{Cl} \\
\text{N} & \quad \text{O-H} \\
\text{4-Cl} & \quad \text{4-Cl} \\
\text{7-Cl} & \quad \text{4-Cl} \\
\text{4-OH} & \quad \text{3-MeO} \\
\text{2-OH} & \quad \text{2-H} \\
\text{3-MeO} & \quad \text{3-H} \\
\text{5-MeO} & \quad \text{5-H} \\
\text{6-MeO} & \quad \text{6-H} \\
\text{7-MeO} & \quad \text{7-H} \\
\text{8-MeO} & \quad \text{8-H} \\
\text{9-MeO} & \quad \text{9-H} \\
\end{align*} \]

The Claisen-Schmidt condensation was conducted in ethanol at room temperature, using drops of 20% sodium hydroxide solution as catalyst. The IR spectrum of compound 5a, for example, showed a characteristic absorption band at 1662 cm\(^{-1}\) corresponding to the stretching vibration of the carbonyl group. Two doublets at 7.81 and 7.45 ppm with \( J = 15.7 \) Hz which correspond to protons H-2" and H-3"...
were observed in the $^1$H-NMR spectrum of compound 5a, confirming the $E$-configuration for the double bond of the $\alpha,\beta$-unsaturated carbonyl moiety.

Chalcones 5 were reacted with hydrazine hydrate, heating to reflux in EtOH, in order to accomplish the synthesis of the $NH$-pyrazolines 6 (Scheme 2), which were obtained in acceptable to excellent yields (71%–96%). Substitution on $N$-1 of pyrazolines 6 was carried out by treating either with acetic anhydride or with formic acid under stirring at room temperature for 10–30 min, to afford the novel $N$-acetyl- or $N$-formylpyrazoline derivatives 7–8 respectively (Scheme 2). These new pyrazolines 6–8, were fully characterized by means of spectroscopic techniques such as FT-IR, $^1$H-NMR, $^{13}$C-NMR and MS (see Experimental Section). As an example, in the IR spectrum of compound 8b, an absorption band is observed at 1,674 cm$^{-1}$ which corresponds to the stretching vibration of the C=O amide functionality and a broad stretching band for the C=N and C=C functionalities is observed at 1591 cm$^{-1}$. In the $^1$H-NMR spectrum the protons on the diastereotopic center C-4', of the pyrazoline ring appears as two double-doublets at $\delta = 3.33$ and 3.98 ppm with $^2J_{AM} = 18.2$, $^3J_{AX} = 5.1$ and $^3J_{MX} = 11.6$ Hz, while the H-5' proton is observed as a double-doublet at 5.64 ppm with $^3J_{MX} = 11.6$ and $^3J_{AX} = 5.1$ Hz. All carbon atoms were completely assigned using DEPT-135, HSQC and HMBC techniques. Finally, mass spectra of compounds 6–8 showed also well-defined molecular ions.

Scheme 2. Synthesis of new $NH$, $N$-acetyl and $N$-formylpyrazolines 6–8.

\[ \text{Scheme 2. Synthesis of new } NH, \text{ } N\text{-acetyl and } N\text{-formylpyrazolines 6–8.} \]
2.2. Anticancer Activity

As a preliminary screening, structures of all new compounds (i.e., 6–8 series) were submitted to the Developmental Therapeutics Program (DTP) at National Cancer Institute (NCI) for evaluation of their anticancer activity against different human tumor cell lines. Thirteen of the submitted structures (i.e., 6b–e; 7b, d, e, f and 8b–f) were selected and subjected to the preliminary evaluation against the 58 tumor cell lines at a single dose of 10 μM after 48 h of incubation. The output from the single dose screening was reported as a mean graph available for analysis by the COMPARE program (data are not shown). The results of this first assay showed that compounds 7b, d, f and 8c, f were active. Then, active compounds passed to a second stage in order to determine their cytostatic activity against the 58 tumor cell lines represented in leukemia, melanoma, lung, colon, brain, breast, ovary, kidney and prostate panels; where the testing results were expressed according to the following three parameters: GI$_{50}$ which is the molar concentration of the compounds required to inhibit the growing of the cell lines to 50% (relative to untreated cells). TGI as the molar concentration that causes total growth inhibition, and LC$_{50}$ which is a parameter of cytotoxicity and reflects the molar concentration needed to kill 50% of the cells [28]. The active compounds were evaluated at five concentration levels (100, 10, 1.0, 0.1, and 0.01 μM) and the test consisted of a 48 h continuous drug exposure protocol using sulforhodamide B (SRB) protein assay to estimate cell growth. Details of this evaluation method, and the complementary information related with the activity pattern over all cell lines, have been published [29–33]. As an outstanding result, compounds 7b, d, f and 8c, f exhibited remarkable activities, with GI$_{50}$ values ranging from $10^{-7}$ to $10^{-6}$ M, nevertheless, a raw comparison of the activities of our obtained compounds 6–8 with respect to the activity reported for the standard drug adriamycin, used by NCI as control, reflects that the activities displayed for our compounds were lower than for the standard drug control as follows: compounds 7d, 7f and 8f displayed activities with GI$_{50}$ values of 1.66, 0.48 and $1.13 \times 10^{-6}$ M respectively, against the SNB-75 cell line (CNS Cancer panel), while this value was $0.07 \times 10^{-6}$ M for the standard drug adriamycin; compound 7b displayed GI$_{50}$ value of $1.40 \times 10^{-6}$ M against BT-549 (breast cancer panel), while the value against the same cell line for adriamycin was $0.23 \times 10^{-6}$ M; finally the compound 8c displayed GI$_{50}$ value of $1.50 \times 10^{-6}$ M against HOP-92 (non-small cell lung panel), while the value was $0.10 \times 10^{-6}$ M for the standard drug adriamycin. The above results suggest that the compounds 7b, d, f and 8c, f are promising structures, of the obtained compounds, for our future drug development antitumor studies. On the other hand, the cytotoxicity associated with the latter compounds, measured as LC$_{50}$ are around 100 μM, for most cell lines, indicating a low toxicity of such compounds for normal human cell lines as required for potential antitumor agents (see Table 1).
Table 1. *In vitro* testing expressed as growth inhibition of cancer cell lines for compounds 7b,d,f and 8c,f and the standard drug adriamycin a.

| Panel/Cell Line | Compounds | Standard Drug |
|-----------------|------------|---------------|
|                 | 7b         | 7d            | 7f            | 8c | 8f | Doxorubicin (Adriamycin), NSC 123127 d |
|                 | GI50 b (µM)| LC50 c (µM)  | GI50 b (µM)  | LC50 c (µM) | GI50 b (µM) | LC50 c (µM) | GI50 b (µM) | LC50 c (µM) | LC50 c (µM) | GI50 b (µM) | LC50 c (µM) |
| Leukemia        |            |               |               |               |              |              |              |              |              |              |              |
| CCRF-CEM        | >100       | >100          | >100          | >100          | >100         | >50          | >100         | >50          | >100         | >50          | >50          |
| HL-60(TB)       | >100       | >100          | >100          | >100          | >100         | >50          | >100         | >50          | >100         | >50          | >50          |
| K-562           | >100       | >100          | >100          | >100          | >100         | >50          | >100         | >50          | >100         | >50          | >50          |
| MOLT-4          | >100       | >100          | >100          | >100          | >100         | >50          | >100         | >50          | >100         | >50          | >50          |
| RPMI-8226       | >100       | >100          | >100          | >100          | >100         | >50          | >100         | >50          | >100         | >50          | >50          |
| SR              | >100       | >100          | >100          | >100          | >100         | >50          | >100         | >50          | >100         | >50          | >50          |
| Non-Small Cell Lung |          |               |               |               |              |              |              |              |              |              |              |
| A549/ATCC       | >100       | >100          | >100          | >100          | 8.87         | >100         | >50          | >100         | >50          | >50          | >50          |
| EK1VX           | >100       | 51.7          | >100          | >100          | 3.67         | >100         | 7.75         | >100         | 1.68         | >100         | 47.97        |
| HOP-62          | 2.36       | >100          | 3.37          | >100          | 4.30         | >100         | >100         | >100         | 1.04         | >100         | >100         |
| HOP-92          | 2.32       | >100          | 3.03          | >100          | 30.1         | >100         | 1.50         | >100         | 9.10         | >100         | >100         |
| NCI-H226        | 2.47       | >100          | 2.78          | >100          | 5.38         | >100         | 5.24         | >100         | 1.81         | >100         | >100         |
| NCI-H23         | >100       | 11.9          | >100          | 2.71          | >100         | 4.82         | >100         | 6.93         | >100         | >100         | >100         |
| NCI-H460        | >100       | >100          | >100          | >100          | 18.5         | >100         | >100         | >100         | >100         | >100         | >100         |
| NCI-H522        | >100       | 7.57          | >100          | >100          | 5.28         | >100         | 2.72         | >100         | >100         | >100         | >100         |
| Colon Cancer    |            |               |               |               |              |              |              |              |              |              |              |
| COLO 205        | >100       | >100          | >100          | >100          | >100         | >50          | >100         | >50          | >100         | >50          | >50          |
| HCC-2998        | >100       | >100          | >100          | >100          | >100         | >50          | >100         | >50          | >100         | >50          | >50          |
| HCT-116         | >100       | 2.61          | >100          | >100          | 0.68         | >100         | >100         | >100         | 2.50         | >100         | >50          |
| HCT-15          | >100       | >100          | >100          | >100          | >100         | >50          | >100         | >50          | >100         | >50          | >50          |
| HT29            | >100       | >100          | >100          | >100          | >100         | >50          | >100         | >50          | >100         | >50          | >50          |
| KM12            | >100       | >100          | >100          | >100          | >100         | >50          | >100         | >50          | >100         | >50          | >50          |
| SW-620          | >100       | >100          | >100          | >100          | >100         | >50          | >100         | >50          | >100         | >50          | >50          |
Table 1. Cont.

| Panel/Cell Line | Compounds | Standard Drug | Doxorubicin (Adriamycin), NSC 123127<sup>d</sup> |
|-----------------|-----------|---------------|-----------------------------------------------|
|                 | 7b        | 7d            | 7f                                           | 8c    | 8f    |
|                 | GI<sub>50</sub><sup>b</sup> | LC<sub>50</sub><sup>c</sup> | GI<sub>50</sub><sup>b</sup> | LC<sub>50</sub><sup>c</sup> | GI<sub>50</sub><sup>b</sup> | LC<sub>50</sub><sup>c</sup> | GI<sub>50</sub><sup>b</sup> | LC<sub>50</sub><sup>c</sup> |
| **CNS Cancer**  |           |               |                                              |       |       |
| SF-268          | >100      | 9.28          | >100                                         | 11.3  | >50   | 0.10  | 30.48 |
| SF-295          | 4.22      | >100          | 5.02                                         | >100  | >50   | 0.10  | 69.98 |
| SF-539          | 2.58      | >100          | 2.40                                         | >100  | >50   | 0.12  | 27.23 |
| SNB-19          | >100      | >100          | 26.8                                         | >100  | >50   | 0.04  | 49.77 |
| SNB-75          | 1.66      | >100          | 1.66                                         | >100  | >50   | 0.04  | 30.62 |
| U251            | 3.09      | >100          | 4.51                                         | >100  | >50   | 0.07  | 3.30  |
| **Melanoma**    |           |               |                                              |       |       |
| LOX IMVI        | >100      | >100          | >100                                         | >100  | >50   | 0.74  | 100.00 |
| MALME-3M        | >100      | >100          | >100                                         | >100  | >50   | 0.12  | 3.97  |
| M14             | >100      | >100          | 93.0                                         | >100  | >50   | 0.18  | 4.05  |
| MDA-MB-435      | >100      | >100          | >100                                         | >100  | >50   | 0.25  | 9.57  |
| SK-MEL-2        | 15.9      | >100          | 7.13                                         | >100  | >50   | 0.17  | 1.06  |
| SK-MEL-28       | >100      | >100          | >100                                         | >100  | >50   | 0.21  | 15.92 |
| SK-MEL-5        | >100      | >100          | >100                                         | >100  | >50   | 0.08  | 0.49  |
| UACC-257        | >100      | >100          | >100                                         | >100  | >50   | 0.14  | 8.15  |
| UACC-62         | >100      | >100          | >100                                         | >100  | >50   | 0.12  | 0.74  |
| **Ovarian Cancer** |       |               |                                              |       |       |
| IGROV1          | >100      | >100          | 6.29                                         | >100  | >50   | 0.17  | 100.00 |
| OVCAR-3         | >100      | >100          | 5.40                                         | >100  | >50   | 0.39  | 84.33 |
| OVCAR-4         | >100      | >100          | >100                                         | >100  | >50   | 0.37  | 74.30 |
| OVCAR-5         | >100      | >100          | >100                                         | >100  | >50   | 0.41  | 100.00 |
| OVCAR-8         | >100      | 3.63          | >100                                         | >100  | >50   | 0.10  | 43.25 |
| NCI/ADR-RES     | >100      | >100          | >100                                         | >100  | >50   | 7.16  | 100.00 |
| SK-OV-3         | >100      | >100          | 6.01                                         | >100  | >50   | 0.22  | 100.00 |
### Table 1. Cont.

| Panel/Cell Line | 7b GI$_{50}$ b (µM) | LC$_{50}$ c (µM) | 7d GI$_{50}$ b (µM) | LC$_{50}$ c (µM) | 7f GI$_{50}$ b (µM) | LC$_{50}$ c (µM) | 8c GI$_{50}$ b (µM) | LC$_{50}$ c (µM) | 8f GI$_{50}$ b (µM) | LC$_{50}$ c (µM) |
|----------------|----------------------|------------------|---------------------|------------------|---------------------|------------------|---------------------|------------------|---------------------|------------------|
| **Renal Cancer** |                     |                  |                     |                  |                     |                  |                     |                  |                     |                  |
| 786-0          | 2.63                 | >100             | 4.52                | >100             | 1.17                | >100             | 11.5                | >100             | 5.07                | >50              |
| A498           | 4.09                 | >100             | 15.2                | >100             | 13.1                | >100             | 6.90                | >100             | 5.73                | >50              |
| ACHN           | >100                 | 4.03             | >100                | 2.76             | >100                | 17.4             | >100                | 4.39             | >50                 | 0.08             |
| Caki-1         | >100 3.32            | >100             | >100                | >100             | >100                | >100             | >100                | >50              | >50                 | 100.00           |
| RXF 393        | >100                 | 5.04             | >100                | 3.57             | >100                | 15.4             | >100                | 4.16             | >50                 | 0.10             |
| SN12C          | 2.63                 | >100             | >100                | >100             | >100                | >100             | >100                | >50              | >50                 | 0.07             |
| UO-31          | >100                 | >100             | >100                | >100             | >100                | >100             | >100                | >50              | >50                 | 0.49             |
| **Prostate Cancer** |                     |                  |                     |                  |                     |                  |                     |                  |                     |                  |
| PC-3           | >100                 | -                | >100                | >100             | >100                | >100             | >100                | >50              | >50                 | 0.32             |
| DU-145         | >100                 | >100             | >100                | >100             | >100                | >100             | >100                | >50              | >50                 | 0.11             |
| **Breast Cancer** |                     |                  |                     |                  |                     |                  |                     |                  |                     |                  |
| MCF7           | >100                 | >100             | 29.4                | >100             | >100                | >100             | >100                | >50              | >50                 | 0.03             |
| MDA-MB-231/ATCC | >100                 | 7.52             | >100                | 11.3             | >100                | 28.5             | >100                | 4.23             | >50                 | 0.51             |
| HS 578T        | 2.94                 | >100             | 5.15                | >100             | 2.60                | >100             | 5.60                | >100             | 1.82                | >50              |
| BT-549         | 1.40                 | >100             | 4.00                | >100             | 3.48                | >100             | 2.24                | >100             | 3.96                | >50              |
| T-47D          | 2.85                 | >100             | 5.54                | >100             | 2.28                | >100             | 10.3                | >100             | 2.90                | >50              |
| MDA-MB-468     | 4.22                 | >100             | 7.43                | >100             | 20.7                | >100             | 2.17                | >100             | 2.59                | >50              |

* Data obtained from NCI’s *in vitro* disease-oriented human tumor cell lines screen [27–29,31,32]; *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 citotoxicity and reflects the molar concentration needed to kill 50% of the cells; *d* The values of activity against human tumor cell lines displayed by adriamycin correspond to the reported by NCI at highest concentration of 10$^{-4}$ M.
3. Experimental Section

3.1. General Information

Commercially available starting materials, reagents and solvents were used as supplied. Microwave reactions were performed in glass vessels (10 mL) using a CEM Discover Focused Microwave Synthesis System™ apparatus, with power output from 0 to 300 W. TLC analyses were performed on Merck silica gel 60 F254 aluminum plates. Melting points were determined in a Büchi melting point apparatus and are uncorrected. IR spectra were performed on a Shimadzu FTIR 8400 spectrophotometer in KBr disks. The 1H- and 13C-NMR spectra were run on a Bruker DPX 400 spectrophotometer operating at 400 MHz and 100 MHz, respectively, using chloroform-d and dimethylsulfoxide-d6 as solvents and tetramethylsilane as internal reference. The mass spectra were obtained on a Hewlett Packard HP Engine-5989 spectrometer (equipped with a direct inlet probe) operating at 70 eV. The elemental analyses were obtained using a Thermo-Finnigan Flash EA1112 CHN (Elemental Microanalysis Ltd, Devon, UK) elemental analyzer.

3.2. Chemistry

3.2.1. General Procedure for the Synthesis of Compound 3 under Microwave Irradiation

A mixture of 4,7-dichloroquinoline 1 (0.5 g, 2.5 mmol), vanillin 2 (0.38 g, 2.5 mmol), potassium carbonate (1 g, 7.2 mmol) in N,N-dimethylformamide was submitted to microwave irradiation for 6 min at a power of 100 W and a temperature of 100 °C. The reaction mixture was cooled and cold water was added. The precipitate of 4-[(7-Chloroquinolin-4-yl)oxy]-3-methoxybenzaldehyde (3) formed was filtered and recrystallized from ethanol. Beige solid; 80% yield; mp: 140–142 °C. FTIR \( \nu (\text{cm}^{-1}) \): 1701 (C=O), 1591 and 1563 (C=N and C=C). 1H-NMR (CDCl3) \( \delta \) ppm 3.82 (s, 3H, OCH3), 6.43 (d, \( J = 5.2 \text{ Hz} \), 1H, H-3), 7.32 (d, \( J = 8.0 \text{ Hz} \), 1H, Ho'), 7.53 (dd, \( J = 9.0 \text{, 2.0 Hz} \), 1H, H-6), 7.55 (dd, \( J = 8.0 \text{, 1.6 Hz} \), 1H, Hm'), 7.59 (d, \( J = 1.6 \text{ Hz} \), 1H, Hm), 8.09 (d, \( J = 2.0 \text{ Hz} \), 1H, H-8), 8.30 (d, \( J = 9.0 \text{ Hz} \), 1H, H-5), 8.65 (d, \( J = 5.2 \text{ Hz} \), 1H, H-2), 9.99 (s, 1H, CHO). 13C-NMR (CDCl3) \( \delta \) ppm 56.1, 104.1, 111.6, 119.5, 122.8, 123.4, 125.2, 127.3, 128.1, 135.2, 136.3, 147.6, 150.3, 152.1, 152.3, 160.9, 190.7. MS (70 eV) \( m/z \) (%): 313 (84, M+), 197 (99), 176 (100), 162 (87), 135 (43), 99 (54). Anal. Calcd. For C17H12ClNO3: C, 65.08; H, 3.86; N, 4.46. Found: C, 64.98; H, 3.89; N, 4.41.

3.2.2. General Procedure for the Synthesis of Chalcones 5a–f

A mixture of aldehyde 3 (300 mg, 1 mmol), the appropriate acetophenone 4 (1 mmol), 20% aq NaOH (0.8 mL) and 95% EtOH (30 mL) was stirred at room temperature for 2 h. The solid formed was filtered and washed with ethanol. No further purification was needed and products were used as such were obtained.

(E)-1-(4-Bromophenyl)-3-[4-[(7-chloroquinolin-4-yl)oxy]-3-methoxyphenyl]prop-2-en-1-one  (5a).

White solid; 93% yield; mp: 177–179 °C. FTIR \( \nu (\text{cm}^{-1}) \): 1662 (C=O), 1605 and 1585 (C=N and C=C). 1H-NMR (CDCl3) \( \delta \) ppm 3.82 (s, 3H, OCH3), 6.43 (d, \( J = 5.3 \text{ Hz} \), 1H, H-3), 7.22 (d, \( J = 8.2 \text{ Hz} \), 1H, Ho'), 7.28 (d, \( J = 1.6 \text{ Hz} \), 1H, Hm), 7.34 (dd, \( J = 8.2 \text{, 1.6 Hz} \), 1H, Hm'), 7.45 (d, \( J = 15.7 \text{, 1H, } =\text{CH} \), 7.53 (dd, \( J = 8.9 \text{, 2.0 Hz} \), 1H, H-6), 7.65 (d, \( J = 8.5 \text{ Hz} \), 2H, Ho'), 7.81 (d, \( J = 15.7 \text{, 1H, } =\text{CH} \), 7.89 (d,
White solid; 95% yield; mp: 168–170 °C. FTIR ν (cm⁻¹): 1661 (C=O), 1603 and 1587 (C=C and C=N). ¹H-NMR (CDCl₃) δ ppm 3.82 (s, 3H, OCH₃), 6.43 (d, J = 5.2 Hz, 1H, H-3). 7.22 (d, J = 8.2 Hz, 1H, Ho'). 7.29 (d, J = 1.7 Hz, 1H, Hm), 7.35 (dd, J = 8.2, 1.7 Hz, 1H, Hm'), 7.45 (d, J = 15.8, 1H, =CH), 7.49 (d, J = 8.5 Hz, 2H, Ho''), 7.53 (dd, J = 8.9, 2.0 Hz, 1H, H-6), 7.81 (d, J = 15.8, 1H, =CH), 7.98 (d, J = 8.5 Hz, 2H, Hm''), 8.09 (d, J = 2.0 Hz, 1H, H-8), 8.33 (d, J = 8.9 Hz, 1H, H-5), 8.64 (d, J = 5.2 Hz, 1H, H-2). ¹³C-NMR (CDCl₃) δ ppm 56.0, 103.7, 112.6, 119.5, 122.0, 123.2, 123.5, 127.1, 128.0, 128.1, 128.7, 130.1, 132.0, 133.8, 136.2, 136.8, 144.2, 144.3, 150.2, 150.0, 152.2, 161.4, 189.2. MS (70 eV) m/z (%): 449 (100, M⁺), 414 (38), 271 (46), 160 (35), 139 (58), 111 (41). Anal. Calcd. For C₂₃H₁₉BrClNO₃: C, 66.68; H, 3.81; N, 3.11. Found: C, 66.35; H, 3.79; N, 3.07.

White solid; 89% yield; mp: 157–159 °C. FTIR ν (cm⁻¹): 1661 (C=O), 1603 and 1583 (C=N and C=C). ¹H-NMR (CDCl₃) δ ppm 3.81 (s, 3H, OCH₃), 6.43 (d, J = 5.3 Hz, 1H, H-3). 7.22 (d, J = 8.2 Hz, 1H, Ho'), 7.30 (d, J = 1.6 Hz, 1H, Hm), 7.34 (dd, J = 8.2, 1.6 Hz, 1H, Hm'), 7.47–7.63 (m, 5H, =CH, H-6, Ho'' and Hp''), 7.80 (d, J = 15.6, 1H, =CH), 8.03 (d, J = 7.3 Hz, 2H, Hm''), 8.08 (d, J = 1.8 Hz, 1H, H-8), 8.33 (d, J = 8.8 Hz, 1H, H-5), 8.64 (d, J = 5.3 Hz, 1H, H-2). ¹³C-NMR (CDCl₃) δ ppm 56.0, 103.7, 112.6, 119.5, 122.0, 122.7, 123.2, 125.7, 127.1, 128.0, 128.6, 132.0, 133.0, 134.0, 136.1, 138.1, 143.8, 144.1, 150.2, 151.9, 152.2, 161.4, 190.3. MS (70 eV) m/z (%): 415 (100, M⁺), 313 (30), 237 (39), 176 (48), 160 (31), 105 (60), 77 (56). Anal. Calcd. For C₂₃H₁₉ClNO₃: C, 72.20; H, 4.36; N, 3.37. Found: C, 72.01; H, 4.34; N, 3.39.

White solid; 62% yield; mp: 190–192 °C. FTIR ν (cm⁻¹): 1655 (C=O), 1606 (C=N and C=C). ¹H-NMR (CDCl₃) δ ppm 3.80 (s, 3H, OCH₃-Ar.C), 3.88 (s, 3H, OCH₃-Ar.A), 6.43 (d, J = 5.3 Hz, 1H, H-3), 6.98 (d, J = 8.9 Hz, 2H, Ho''), 7.20 (d, J = 8.0 Hz, 1H, Ho'), 7.28 (d, J = 1.6 Hz, 1H, Hm), 7.33 (dd, J = 8.0, 1.6 Hz, 1H, Hm'), 7.50–7.54 (m, 2H, =CH-H), 7.79 (d, J = 15.6, 1H, =CH), 8.04 (d, J = 8.9 Hz, 2H, Hm''), 8.07 (d, J = 1.9 Hz, 1H, H-8), 8.33 (d, J = 8.9 Hz, 1H, H-5), 8.63 (d, J = 5.3 Hz, 1H, H-2). ¹³C-NMR (CDCl₃) δ ppm 55.5, 56.0, 103.7, 112.6, 114.0, 119.5, 121.8, 122.8, 123.5, 127.1, 128.0, 130.7, 131.1, 134.3, 136.1, 142.9, 143.9, 150.2, 151.9, 152.2, 161.4, 163.6, 188.4. MS (70 eV) m/z (%): 445 (13, M⁺), 313 (85), 176 (98), 135 (100). Anal. Calcd. For C₂₆H₂₆ClNO₄: C, 70.03; H, 4.52; N, 3.14. Found: C, 70.00; H, 4.50; N, 3.18.

White solid; 58% yield; mp: 200–202 °C. FTIR ν (cm⁻¹): 1650 (C=O), 1586 (C=N and C=C). ¹H-NMR (DMSO-d₆) δ ppm 3.79 (s, 3H, OCH₃-Ar.A), 3.83 (s, 3H, OCH₃-Ar.C), 3.92 (s, 6H, OCH₃ × 2-Ar.A), 6.55 (d, J = 5.3 Hz, 1H, H-3), 7.42 (d, J = 8.3 Hz, 1H, Ho'), 7.45 (s, 2H, Ho''), 7.68–7.84 (m,
4H, H-6, Hm, Hm', =CH), 7.98 (d, J = 15.8, 1H, =CH), 8.09 (d, J = 2.0 Hz, 1H, H-8), 8.37 (d, J = 8.9 Hz, 1H, H-5), 8.70 (d, J = 5.3 Hz, 1H, H-2). $^{13}$C-NMR (DMSO-d$_6$) δ ppm 56.1, 56.5, 61.0, 103.6, 106.4, 113.0, 119.5, 121.6, 122.4, 123.2, 123.5, 127.1, 128.0, 133.3, 134.1, 136.1, 142.9, 143.6, 144.1, 150.2, 151.9, 152.2, 153.3, 161.4, 189.0. MS (70 eV) m/z (%): 505 (100, M$^+$), 490 (97), 195 (63), 160 (32). Anal. Calcd. For C$_{23}$H$_{24}$ClNO$_3$: C, 66.47; H, 4.78; N, 2.77. Found: C, 66.42; H, 4.71; N, 2.69.

(E)-3-[4-((7-Chloroquinolin-4-yl)oxy)-3-methoxyphenyl]I-(p-toly1)prop-2-en-1-one (5f). White solid; 85% yield; mp: 182–183 °C. FTIR v (cm$^{-1}$): 1658 (C=O), 1604 and 1564 (C=N and C=C). $^1$H-NMR (CDCl$_3$) δ ppm 2.43 (s, 3H, CH$_3$), 3.81 (s, 3H, OCH$_3$), 6.43 (d, J = 5.3 Hz, 1H, H-3), 7.21 (d, J = 8.0 Hz, 1H, Ho$^b$), 7.29 (d, J = 2.0 Hz, 1H, Hm), 7.30–7.34 (m, 3H, Ho$^c$, Hm$^c$), 7.47–7.57 (m, 2H, H-6, =CH), 7.79 (d, J = 15.6, 1H, =CH), 7.94 (d, J = 8.3 Hz, 2H, Hm$^c$), 8.08 (d, J = 1.8 Hz, 1H, H-8), 8.33 (d, J = 9.0 Hz, 1H, H-5), 8.63 (d, J = 5.3 Hz, 1H, H-2). $^{13}$C-NMR (CDCl$_3$) δ ppm 21.7, 56.0, 80.3, 112.6, 119.5, 121.9, 122.7, 123.2, 123.5, 127.1, 128.0, 128.7, 129.4, 134.2, 135.5, 136.1, 143.3, 143.9, 144.0, 150.2, 151.9, 152.2, 161.4, 189.8. MS (70 eV) m/z (%): 429 (61, M$^+$), 313 (76), 176 (100), 135 (32), 119 (64), 91 (58). Anal. Calcd. For C$_{26}$H$_{26}$ClNO$_3$: C, 72.64; H, 4.69; N, 3.26. Found: C, 72.59; H, 4.59; N, 3.33.

3.2.3. General Procedure for the Synthesis of the NH-Pyrazolines 6a–f

A mixture of chalcone 5 (100 mg, 0.20 mmol), hydrazine hydrate (0.26 mmol) in absolute ethanol (15 mL) was heated under reflux for 1 h until complete consumption of the chalcone (TLC control). The solid formed was filtered and washed with cold ethanol/water (1:0.5) mixture. No further purification was required.

4-(4-(4-((4-Bromophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenoxy)-7-chloroquinoline (6a). White solid; 96% yield; mp: 230–232 °C. FTIR v (cm$^{-1}$): 3331 (NH), 1611 and 1587 (C=N and C=C). $^1$H-NMR (DMSO-d$_6$) δ ppm 2.95 (dd, J = 16.3, 11.2 Hz, 1H, H-4'a), 3.51 (dd, J = 16.3, 11.2 Hz, 1H, H-4'b), 3.73 (s, 3H, OCH$_3$), 4.96 (m, 1H, H-5'), 6.44 (d, J = 5.3 Hz, 1H, H-3), 7.09 (dd, J = 8.3, 1.5 Hz, 1H, Hm$^c$), 7.29 (d, J = 8.3 Hz, 1H, Ho$^b$), 7.32 (d, J = 1.5 Hz, 1H, Hm), 7.51–7.62 (m, 4H, Ho$^a$, Hm$^c$), 7.68 (dd, J = 8.9, 2.0 Hz, 1H, H-6), 8.07 (d, J = 2.0 Hz, 1H, H-8), 8.35 (d, J = 8.9 Hz, 1H, H-5), 8.68 (d, J = 5.3 Hz, 1H, H-2), Not observed (s, 1H, NH). $^{13}$C-NMR (DMSO-d$_6$) δ ppm 41.6, 56.8, 63.8, 104.6, 113.6, 119.7, 120.1, 122.7, 124.5, 126.1, 127.1, 127.9, 129.4, 131.2, 132.6, 135.2, 138.1, 141.6, 143.5, 149.1, 150.2, 151.2, 153.2. MS (70 eV) m/z (%): 507 (38, M$^+$), 509 (100), 495 (65), 414 (31), 312 (81), 285 (29), 183 (62), 176 (46), 155 (32), 135 (39), 99 (29), 69 (52). Anal. Calcd. For C$_{28}$H$_{19}$BrClN$_3$O$_2$: C, 59.02; H, 3.76; N, 8.26. Found: C, 59.13; H, 3.80; N, 8.31.

7-Chloro-4-(4-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenoxy)quinoline (6b). White solid; 95% yield; mp: 223–225 °C. FTIR v (cm$^{-1}$): 3333 (NH), 1611 and 1589 (C=N and C=C). $^1$H-NMR (DMSO-d$_6$) δ ppm 2.97 (dd, J = 16.3, 10.8 Hz, 1H, H-4'a), 3.52 (dd, J = 16.3, 10.8 Hz, 1H, H-4'b), 3.74 (s, 3H, OCH$_3$), 4.98 (m, 1H, H-5'), 6.49 (d, J = 5.1 Hz, 1H, H-3), 7.10 (dd, J = 8.0, 1.8 Hz, 1H, Hm$^c$), 7.26 (d, J = 8.0 Hz, 1H, Ho$^c$), 7.30 (d, J = 1.8 Hz, 1H, Hm), 7.43 (d, J = 8.5 Hz, 2H, Hm$^c$), 7.56 (dd, J = 9.0, 2.0 Hz, 1H, H-6), 7.66 (d, J = 8.5 Hz, 2H, Hm$^c$), 8.05 (d, J = 2.0 Hz, 1H, H-8), 8.36 (d, J = 9.0 Hz, 1H, H-5), 8.68 (d, J = 5.1 Hz, 1H, H-2), Not observed (s, 1H, NH). $^{13}$C-NMR (DMSO-d$_6$) δ ppm 41.4, 56.8, 65.2, 103.4, 112.2, 114.2, 119.1, 119.7, 120.3, 122.5, 124.3, 125.8, 126.0, 127.1, 127.4,
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127.7, 128.0, 128.5, 129.2, 133.3, 134.0, 135.3, 153.1. MS (70 eV) m/z (%): 463 (92, M⁺), 449 (64), 312 (87), 298 (38), 285 (37), 271 (34), 177 (52), 162 (40), 151 (98), 139 (100), 111 (40). Anal. Calcd. For C₂₅H₁₇Cl₂N₂O₂: C, 64.66; H, 4.12; N, 9.05. Found: C, 64.57; H, 4.09; N, 9.11.

7-Chloro-4-(2-methoxy-4-(3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)quinoline (6e). White solid; 81% yield; mp: 178–179 °C. FTIR (v (cm⁻¹)): 3345 (NH), 1612 and 1589 (C=N and C=C). ¹H-NMR (DMSO-d₆) δ ppm 2.99 (dd, J = 16.1, 10.7 Hz, 1H, H-4'a), 3.53 (dd, J = 16.1, 10.7 Hz, 1H, H-4'b), 3.75 (s, 3H, OCH₃), 4.98 (m, 1H, H-5'), 6.51 (d, J = 4.9 Hz, 1H, H-3'), 7.12 (d, J = 8.2 Hz, 1H, Hm'), 7.24 (d, J = 8.2 Hz, 1H, H'), 7.29–7.43 (m, 4H, Hm, Hm'', Hp''), 7.59–7.70 (m, 3H, H', H-6), 8.05 (s, 1H, H-8), 8.36 (d, J = 8.8 Hz, 1H, H-5), 8.68 (d, J = 4.9 Hz, 1H, H-2), Not observed (s, 1H, NH). ¹³C-NMR (DMSO-d₆) δ ppm 41.2, 56.7, 63.9, 104.4, 112.6, 113.2, 119.0, 119.7, 120.1, 122.9, 124.2, 125.8, 126.0, 127.1, 127.8, 127.9, 128.5, 129.2, 133.3, 134.5, 135.4, 153.2. MS (70 eV) m/z (%): 431 (5, M⁺), 327 (64), 312 (100), 176 (40). Anal. Calcd. For C₂₅H₂₀Cl₂N₂O₂: C, 69.85; H, 4.69; N, 9.77. Found: C, 69.71; H, 4.75; N, 9.69.

7-Chloro-4-(2-methoxy-4-(3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)quinoline (6d). White solid; 76% yield; mp: 214–216 °C. FTIR (v (cm⁻¹)): 3215 (NH), 1568 and 1503 (C=N and C=C). ¹H-NMR (DMSO-d₆) δ ppm 2.91 (dd, J = 16.3, 10.9 Hz, 1H, H-4'a), 3.49 (dd, J = 16.3, 10.9 Hz, 1H, H-4'b), 3.73 (s, 3H, OCH₃-Ar-C), 3.78 (s, 3H, OCH₃-Ar-A), 4.90 (m, 1H, H-5'), 6.45 (d, J = 5.2 Hz, 1H, H-3), 6.96 (d, J = 8.8 Hz, 2H, H''), 7.10 (dd, J = 8.2, 1.5 Hz, 1H, Hm'), 7.28 (d, J = 8.2 Hz, 1H, H''), 7.33 (d, J = 1.5 Hz, 1H, Hm), 7.59 (d, J = 8.8 Hz, 2H, Hm''), 7.67 (dd, J = 8.9, 2.0 Hz, 1H, H-6), 8.07 (d, J = 2.0 Hz, 1H, H-8), 8.35 (d, J = 8.9 Hz, 1H, H-5), 8.68 (d, J = 5.2 Hz, 1H, H-2), Not observed (s, 1H, NH). ¹³C-NMR (DMSO-d₆) δ ppm 40.5, 54.7, 55.3, 62.9, 103.1, 111.6, 113.5, 118.4, 119.0, 122.1, 123.4, 125.4, 126.3, 126.5, 126.9, 134.3, 139.7, 142.2, 148.5, 149.1, 150.5, 152.4, 158.9, 160.5. MS (70 eV) m/z (%): 459 (6, M⁺), 461 (8), 135 (100). Anal. Calcd. For C₂₆H₂₂ClN₂O₃: C, 67.90; H, 4.82; N, 9.14. Found: C, 67.85; H, 4.86; N, 9.09.

7-Chloro-4-(2-methoxy-4-(3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)-quinoline (6e). White solid; 71% yield; mp: 226–227 °C. FTIR (v (cm⁻¹)): 3216 (NH), 1572 and 1507 (C=N and C=C). ¹H-NMR (DMSO-d₆) δ ppm Not observed (dd, 1H, H-4'a, overlapped with water signal), 3.53 (dd, J = 16.1, 10.7 Hz, 1H, H-4'b), 3.75 (s, 3H, OCH₃-Ar-C), 3.76 (s, 3H, OCH₃-Ar-A), 3.84 (s, 6H, OCH₃ × 2-Ar-A), 4.96 (m, 1H, H-5'), 6.50 (d, J = 5.0 Hz, 1H, H-3), 6.97 (s, 2H, H''), 7.12 (d, J = 8.2 Hz, 1H, Hm'), 7.25 (d, J = 8.2 Hz, 1H, H''), 7.31 (s, 3I, Hm), 7.65 (dd, J = 8.9, 1.4 Hz, 1H, H-6), 8.05 (d, J = 1.4 Hz, 1H, H-8), 8.36 (d, J = 8.9 Hz, 1H, H-5), 8.68 (d, J = 5.0 Hz, 1H, H-2), Not observed (s, 1H, NH). ¹³C-NMR (DMSO-d₆) δ ppm 41.4, 56.7, 56.9, 63.9, 104.4, 104.8, 105.0, 113.3, 119.7, 120.0, 122.9, 124.2, 124.9, 127.1, 127.9, 129.0, 129.4, 137.8, 139.6, 141.6, 143.1, 149.4, 153.2, 153.6, 161.2. MS (70 eV) m/z (%): 519 (2, M⁺), 521 (8), 313 (62), 210 (55), 195 (100), 176 (66). Anal. Calcd. For C₂₈H₂₆ClN₃O₅: C, 64.68; H, 5.04; N, 8.08. Found: C, 64.59; H, 5.09; N, 8.13.

7-Chloro-4-(2-methoxy-4-(3-(p-tolyl)-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)quinoline (6f). White solid; 80% yield; mp: 179–181 °C. FTIR (v (cm⁻¹)): 3335 (NH), 1601 and 1574 (C=N and C=C). ¹H-NMR (DMSO-d₆) δ ppm 2.33 (s, 3H, CH₃), 2.95 (dd, J = 16.0, 10.5 Hz, 1H, H-4'a), 3.50 (dd, J = 16.0, 10.5 Hz, 1H, H-4'b), 3.74 (s, 3H, OCH₃), 4.94 (m, 1H, H-5'), 6.50 (d, J = 4.6 Hz, 1H, H-3), 7.11 (d, J = 8.2 Hz,
1H, Hm'), 7.20 (d, J = 7.5 Hz, 2H, Ho''), 7.24 (d, J = 8.2 Hz, 1H, Ho'), 7.31 (s, 2H, Hm, NH), 7.55 (d, J = 7.5 Hz, 2H, Hm''), 7.65 (d, J = 8.8 Hz, 1H, H-6), 8.05 (s, 1H, H-8), 8.36 (d, J = 8.8 Hz, 1H, H-5), 8.68 (d, J = 4.6 Hz, 1H, H-2). 13C-NMR (DMSO-d6) δ ppm 21.2, 56.7, 63.8, 104.4, 113.3, 119.7, 120.0, 122.8, 124.2, 126.0, 127.1, 127.9, 129.4, 131.2, 132.6, 135.3, 138.1, 141.6, 143.1, 149.5, 150.3, 151.7, 153.2, 161.6. MS (70 eV) m/z (%): 443 (3, M'), 445 (24), 176 (42), 119 (100), 91 (58). Anal. Caled. For C28H22ClN3O2: C, 70.34; H, 5.00; N, 9.47. Found: C, 70.07; H, 5.03; N, 9.50.

3.2.4. General Procedure for the Synthesis of the N-Acetylpyrazolines 7a–f

A mixture of the NH-pyrazoline 6 (50 mg, 0.10 mmol), acetic anhydride (2 mL) and pyridine (3 drops) was stirred at room temperature for 10 min until complete consumption of the NH-pyrazoline (TLC control). Then, water (3 mL) was added and the resulting precipitate was collected by filtration, washed with water and recrystallized from ethanol.

1-(3-(4-Bromophenyl)-5-[4-(7-chloroquinolin-4-ylxoy)-3-methoxyphenyl]-4,5-dihydro-1H-pyrazol-1-yl)ethanone (7a). White solid; 91% yield; mp: 244–245 °C. FTIR ν (cm⁻¹): 1665 (C=O), 1585 (C=N and C=C). 1H-NMR (DMSO-d6) δ ppm Not observed (dd, 1H, H-4a, overlapped with water signal). 2.35 (s, 3H, CH3), 3.71 (s, 3H, OCH3), 3.91 (dd, J = 18.1, 11.8 Hz, 1H, H-4b), 5.64 (dd, J = 11.8, 4.8 Hz, 1H, H-5), 6.43 (d, J = 5.3 Hz, 1H, H-3), 6.85 (dd, J = 8.2, 1.8 Hz, 1H, Hm''), 7.14 (d, J = 1.8 Hz, 1H, Hm), 7.26 (d, J = 8.2 Hz, 1H, Ho'), 7.65–7.71 (m, 3H, H-6, Ho''), 7.73–7.77 (m, 2H, Hm''), 8.07 (d, J = 2.0 Hz, 1H, H-8), 8.33 (d, J = 8.8 Hz, 1H, H-5), 8.67 (d, J = 5.3 Hz, 1H, H-2). 13C-NMR (DMSO-d6) δ ppm 21.9, 42.2, 56.0, 60.0, 100.0, 103.7, 111.4, 118.0, 119.0, 123.0, 123.3, 123.9, 127.0, 127.5, 128.8, 130.5, 131.9, 134.9, 140.4, 140.8, 149.7, 151.3, 153.0, 153.5, 167.9. MS (70 eV) m/z (%): 549 (34, M'), 551 (44), 509 (63), 368 (56), 285 (32), 43 (100). Anal. Caled. For C27H21BrClN3O3: C, 58.87; H, 3.84; N, 7.63. Found: C, 58.35; H, 3.79; N, 7.70.

1-(3-(4-Chlorophenyl)-5-[4-(7-chloroquinolin-4-ylxoy)-3-methoxyphenyl]-4,5-dihydro-1H-pyrazol-1-yl)ethanone (7b). White solid; 91% yield; mp: 236–238 °C. FTIR ν (cm⁻¹): 1666 (C=O), 1595 (C=N and C=C). 1H-NMR (DMSO-d6) δ ppm 2.35 (s, 3H, CH3), 3.25 (dd, J = 17.8, 4.4 Hz, 1H, H-4a), 3.72 (s, 3H, OCH3), 3.91 (dd, J = 17.8, 11.7 Hz, 1H, H-4b), 5.65 (dd, J = 11.7, 4.4 Hz, 1H, H-5), 6.47 (d, J = 4.5 Hz, 1H, H-3), 6.88 (d, J = 7.5 Hz, 1H, Hm'), 7.13 (s, 1H, Hm), 7.23 (d, J = 7.5 Hz, 1H, Ho'), 7.52 (d, J = 8.0 Hz, 2H, Ho''), 7.65 (d, J = 8.8 Hz, 1H, H-6), 7.81 (d, J = 8.0 Hz, 2H, Hm''), 8.05 (s, 1H, H-8), 8.33 (d, J = 8.8 Hz, 1H, H-5), 8.67 (d, J = 4.5 Hz, 1H, H-2). 13C-NMR (DMSO-d6) δ ppm 22.1, 42.6, 56.7, 60.2, 104.4, 112.6, 118.8, 119.7, 123.1, 124.2, 127.1, 127.2, 127.9, 128.8, 129.3, 135.1, 135.4, 141.7, 142.0, 150.3, 151.8, 153.2, 153.6, 161.4, 168.2. MS (70 eV) m/z (%): 505 (33, M'), 463 (55), 368 (41), 285 (31), 179 (54), 43 (100). Anal. Caled. For C27H21Cl2N3O3: C, 64.04; H, 4.18; N, 8.30. Found: C, 63.97; H, 4.00; N, 8.36.

1-(5-[4-(7-Chloroquinolin-4-ylxoy)-3-methoxyphenyl]-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethanone (7c). White solid; 90% yield; mp: 240–241 °C. FTIR ν (cm⁻¹): 1663 (C=O), 1597 and 1570 (C=N and C=C). 1H-NMR (DMSO-d6) δ ppm 2.36 (s, 3H, CH3), 3.20–3.30 (m, 1H, H-4a), 3.72 (s, 3H, OCH3), 3.93 (dd, J = 16.6, 12.6 Hz, 1H, H-4b), 5.61–5.67 (m, 1H, H-5'), 6.49 (s, 1H, H-3), 6.89 (d, J = 6.9 Hz, 1H, Hm'), 7.13 (s, 1H, Hm), 7.22 (d, J = 6.9 Hz, 1H, Ho'), 7.48 (s, 3H, Ho'', Hp'').
1-(5-[4-(7-Chloroquinolin-4-yl)-3-methoxyphenyl]-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethaneone (7d). White solid; 75% yield; mp: 238–239 °C. FTIR ν (cm⁻¹): 1663 (C=O), 1607 and 1576 (C=N and C=C). ¹H-NMR (DMSO-d₆) δ ppm 2.34 (s, 3H, CH₃), 3.22 (dd, J = 17.8, 2.8 Hz, 1H, H-4'a), 3.72 (s, 3H, OCH₃-Ar.C), 3.81–3.94 (m, 4H, OCH₂-Ar.A-H-4'b), 5.58–5.64 (m, 1H, H-5'), 6.49 (d, J = 2.8 Hz, 1H, H-3), 6.88 (d, J = 7.4 Hz, 1H, Hm''), 7.03 (d, J = 7.4 Hz, 2H, Ho''), 7.12 (s, 1H, Hm''), 7.22 (d, J = 7.4 Hz, 1H, Ho') 7.64 (d, J = 8.2 Hz, 1H, H-6) 7.75 (d, J = 7.4 Hz, 2H, Hm'') 8.04 (s, 1H, H-8) 8.33 (d, J = 8.2 Hz, 1H, H-5) 8.67 (d, J = 2.8 Hz, 1H, H-2). ¹³C-NMR (DMSO-d₆) δ ppm 22.1, 42.8, 56.0, 56.7, 59.9, 100.2, 104.4, 112.5, 114.9, 118.7, 119.7, 123.1, 124.2, 124.5, 127.1, 127.9, 128.7, 135.3, 141.6, 142.3, 150.6, 151.8, 153.2, 154.4, 161.4, 168.3. MS (70 eV) m/z (%): 501 (74, M⁺), 459 (67), 175 (40), 134 (42), 43 (100). Anal. Calcd. For C₃₂H₂₄ClN₃O₃: C, 67.00; H, 4.82; N, 8.37. Found: C, 67.15; H, 4.87; N, 8.29.

1-(5-[4-(7-Chloroquinolin-4-yl)-3-methoxyphenyl]-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethaneone (7e). White solid; 73% yield; mp: 223–224 °C. FTIR ν (cm⁻¹): 1659 (C=O), 1603 and 1572 (C=N and C=C). ¹H-NMR (DMSO-d₆) δ ppm 2.36 (s, 3H, CH₃) 3.28 (dd, J = 18.1, 4.0 Hz, 1H, H-4'a) 3.73 (s, 3H, OCH₃-Ar.C) 3.76 (s, 3H, OCH₃-Ar.A) 3.82–3.96 (m, 7H, OCH₃×2-Ar.A and H-4'b) 5.64 (dd, J = 11.3, 4.0 Hz, 1H, H-5') 6.49 (d, J = 4.8 Hz, 1H, H-3) 6.89 (d, J = 8.2 Hz, 1H, Hm'') 7.06–7.16 (m, 3H, Hm and Ho'') 7.23 (d, J = 8.2 Hz, 1H, Ho'') 7.65 (d, J = 8.9 Hz, 1H, H-6) 8.05 (s, 1H, H-8) 8.34 (d, J = 8.9 Hz, 1H, H-5) 8.68 (d, J = 4.8 Hz, 1H, H-2). ¹³C-NMR (DMSO-d₆) δ ppm 22.1, 42.8, 56.7, 57.1, 60.1, 60.7, 104.4, 106.0, 112.5, 118.7, 119.7, 123.1, 124.2, 127.1, 127.9, 135.3, 141.2, 141.6, 142.2, 150.3, 151.8, 153.2, 153.8, 154.5, 161.5, 168.1. MS (70 eV) m/z (%): 561 (100, M⁺), 519 (73), 504 (32), 285 (19), 236 (36), 97 (33), 83 (36), 57 (53), 43 (52). Anal. Calcd. For C₃₀H₂₆ClN₃O₆: C, 64.11; H, 5.02; N, 7.48. Found: C, 64.38; H, 5.10; N, 7.56.

1-(5-[4-(7-Chloroquinolin-4-yl)-3-methoxyphenyl]-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)ethaneone (7f). White solid; 85% yield; mp: 250–251 °C. FTIR ν (cm⁻¹): 1663 (C=O), 1601 and 1576 (C=N and C=C). ¹H-NMR (DMSO-d₆) δ ppm 2.35 (s, 3H, CH₃-Ar.A), 2.37 (s, 3H, CH₃-Ar.B) 3.22 (dd, J = 17.7, 3.7 Hz, 1H, H-4'a) 3.72 (s, 3H, OCH₃) 3.90 (dd, J = 17.7, 9.6 Hz, 1H, H-4'b) 5.62 (dd, J = 9.6, 3.7 Hz, 1H, H-5') 6.49 (d, J = 4.4 Hz, 1H, H-3) 6.88 (d, J = 7.4 Hz, 1H, Hm'') 7.12 (s, 1H, Hm') 7.22 (d, J = 7.4 Hz, 1H, Ho') 7.29 (d, J = 6.9 Hz, 2H, Ho'') 7.65 (d, J = 8.4 Hz, 1H, H-6) 7.70 (d, J = 6.9 Hz, 2H, Hm'') 8.04 (s, 1H, H-8) 8.34 (d, J = 8.4 Hz, 1H, H-5) 8.67 (d, J = 4.4 Hz, 1H, H-2). ¹³C-NMR (DMSO-d₆) δ ppm 21.3, 22.1, 42.7, 56.7, 59.9, 104.5, 112.5, 118.7, 119.7, 123.1, 124.2, 126.6, 127.3, 127.9, 129.6, 129.4, 135.3, 140.6, 141.6, 142.2, 150.3, 151.8, 153.2, 154.6, 161.4, 168.1. MS (70 eV) m/z (%): 485 (86, M⁺), 443 (100), 159 (68), 118 (43), 91 (29), 43 (44). Anal. Calcd. For C₂₈H₂₄ClN₃O₃: C, 69.20; H, 4.98; N, 8.65. Found: C, 69.27; H, 4.88; N, 8.31.
3.2.5. General Procedure for the Synthesis of the N-Formylpyrazolines 8a–f

A mixture of the NH-pyrazoline 6 (50 mg, 0.10 mmol) and formic acid (2 mL) was stirred at room temperature for 30 min until complete consumption of the NH-pyrazoline (TLC control). Then, the adding of crushed ice to the solution precipitated a solid which was filtered, washed with water and recrystallized from ethanol.

3-(4-Bromophenyl)-5-[4-(7-chloroquinolin-4-yloxy)-3-methoxyphenyl]-4,5-dihydro-1H-pyrazole-1-carbaldehyde (8a). White solid; 94% yield; mp: 230–231 °C. FTIR v (cm⁻¹): 1674 (C=O), 1593 (C=N and C=C). 1H-NMR (DMSO-d6) δ ppm 3.28–3.36 (m, 1H, H-4′a), 3.73 (s, 3H, OCH3), 3.91–4.03 (m, 1H, H-4′b), 5.60–5.66 (m, 1H, H-5′), 6.50 (d, J = 1.9 Hz, 1H, H-3), 6.96 (d, J = 6.7 Hz, 1H, Hm'), 7.18 (s, 1H, Hm), 7.25 (d, J = 6.7 Hz, 1H, Ho'), 7.59–7.82 (m, 5H, Hm", H-6, Ho''), 8.05 (s, 1H, H-8), 8.34 (d, J = 8.3 Hz, 1H, H-5), 8.68 (d, J = 1.9 Hz, 1H, H-2), 8.95 (s, 1H, CHO). 13C-NMR (DMSO-d6) δ ppm 42.2, 55.8, 58.6, 99.5, 103.6, 111.4, 118.3, 118.8, 122.9, 123.8, 124.0, 126.8, 127.4, 128.7, 131.8, 134.8, 137.4, 140.5, 150.3, 151.2, 152.9, 155.3, 159.9, 160.8. MS (70 eV) m/z (%): 535 (47, M⁺), 537 (64), 354 (25), 197 (30), 176 (92), 85 (66), 83 (100), 47 (33). Anal. Calcd. For C26H19BrClN3O3: C, 58.17; H, 3.57; N, 7.83. Found: C, 58.09; H, 3.55; N, 7.87.

3-(4-Chlorophenyl)-5-[4-(7-chloroquinolin-4-yloxy)-3-methoxyphenyl]-4,5-dihydro-1H-pyrazole-1-carbaldehyde (8b). White solid; 92% yield; mp: 206–209 °C. FTIR v (cm⁻¹): 1674 (C=O), 1591 (C=N and C=C). 1H-NMR (DMSO-d6) δ ppm 3.33 (dd, J = 18.2, 5.1 Hz, 1H, H-4′a), 3.73 (s, 3H, OCH3), 3.98 (dd, J = 18.2, 11.6 Hz, 1H, H-4′b), 5.64 (dd, J = 11.6, 5.1 Hz, 1H, H-5′), 6.49 (d, J = 5.0 Hz, 1H, H-3), 6.95 (d, J = 8.0 Hz, 1H, Hm'), 7.18 (s, 1H, Hm), 7.25 (d, J = 8.0 Hz, 1H, Ho'), 7.53 (d, J = 8.3 Hz, 2H, Ho''), 7.65 (d, J = 8.8 Hz, 1H, H-6), 7.82 (d, J = 8.3 Hz, 2H, Hm''), 8.05 (s, 1H, H-8), 8.34 (d, J = 8.8 Hz, 1H, H-5), 8.67 (d, J = 5.0 Hz, 1H, H-2), 8.94 (s, 1H, CHO). 13C-NMR (DMSO-d6) δ ppm 42.3, 55.9, 58.6, 99.5, 103.6, 111.5, 118.2, 118.8, 122.9, 123.8, 126.8, 127.4, 128.5, 128.9, 129.7, 134.8, 135.2, 140.5, 149.5, 151.2, 152.9, 155.2, 160.0, 160.8. MS (70 eV) m/z (%): 491 (95, M⁺), 311 (33), 176 (100), 153 (61), 138 (54), 85 (38), 83 (59), 43 (25). Anal. Calcd. For C26H19Cl2N3O3: C, 63.43; H, 3.89; N, 8.53. Found: C, 63.11; H, 3.96; N, 8.42.

5-[4-(7-Chloroquinolin-4-yloxy)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbaldehyde (8c). White solid; 88% yield; mp: 208–210 °C. FTIR v (cm⁻¹): 1676 (C=O), 1585 (C=N and C=C). 1H-NMR (DMSO-d6) δ ppm Not observed (dd, 1H, H-4′a, overlapped with water signal), 3.72 (s, 3H, OCH3), 3.99 (dd, J = 18.1, 11.8 Hz, 1H, H-4′b), 5.64 (dd, J = 11.8, 5.0 Hz, 1H, H-5′), 6.44 (d, J = 5.3 Hz, 1H, H-3), 6.92 (dd, J = 8.2, 1.8 Hz, 1H, Hm'), 7.19 (d, J = 1.8 Hz, 1H, Hm), 7.28 (d, J = 8.2 Hz, 1H, Ho'), 7.46–7.53 (m, 3H, Ho'', Hp''), 7.67 (dd, J = 8.8, 2.1 Hz, 1H, H-6), 7.79–7.85 (m, 2H, Hm''), 8.07 (d, J = 2.1 Hz, 1H, H-8), 8.34 (d, J = 8.8 Hz, 1H, H-5), 8.67 (d, J = 5.3 Hz, 1H, H-2), 8.97 (s, 1H, CHO). 13C-NMR (DMSO-d6) δ ppm 41.6, 55.5, 57.9, 103.2, 111.5, 117.9, 118.4, 122.0, 122.9, 126.0, 126.7, 127.9, 130.0, 130.2, 134.1, 139.7, 140.7, 140.5, 149.0, 150.7, 151.9, 155.4, 159.9, 160.2. MS (70 eV) m/z (%): 457 (100, M⁺), 176 (72), 145 (59), 119 (65), 104 (55). Anal. Calcd. For C26H20ClN3O3: C, 68.20; H, 4.40; N, 9.18. Found: C, 68.12; H, 4.52; N, 9.03.
5-[4-(7-Chloroquinolin-4-yl oxy)-3-methoxyphenyl]-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde (8d). White solid; 86% yield; mp: 239–240 °C. FTIR ν (cm⁻¹): 1670 (C=O), 1603 (C=N and C=C). ¹H-NMR (DMSO-d₆) δ ppm Not observed (dd, J₁ = 11.7, 4.1 Hz, 1H, H-5'); 6.44 (J₁ = 4.9 Hz, 1H, H-3) 6.90 (J₂ = 8.0 Hz, 1H, Hm') 7.03 (J₂ = 8.3 Hz, 2H, Hο') 7.18 (J₁ = 11.8, 5.0 Hz, 1H, H-5) 6.44 (J₁ = 5.3 Hz, 1H, H-3) 6.91 (J₁ = 8.3, 1.3 Hz, 1H, Hm') 7.09 (s, 2H, Hο') 7.18 (J₁ = 1.3 Hz, 1H, Hm) 7.30 (J₁ = 8.3 Hz, 1H, Hο') 7.67 (J₂ = 9.0, 1.8 Hz, 1H, H-6) 8.07 (J₂ = 1.8 Hz, 1H, H-8) 8.34 (J₁ = 9.0 Hz, 1H, H-5) 8.68 (J₁ = 5.3 Hz, 1H, H-2) 8.97 (s, 1H, CHO) ¹³C-NMR (DMSO-d₆) δ ppm 42.4, 55.9, 58.2, 103.6, 111.4, 114.3, 118.1, 118.8, 122.9, 123.8, 126.0, 126.8, 127.4, 128.4, 129.7, 134.8, 139.7, 140.8, 149.6, 150.7, 152.9, 155.9, 159.7, 161.2. MS (70 eV) m/z (%): 487 (100, M⁺), 175 (70), 149 (87), 134 (99). Anal. Calcd. For C₂₇H₂₂ClN₃O₆: C, 66.46; H, 4.54; N, 8.61. Found: C, 66.26; H, 4.56; N, 8.58.

5-[4-(7-Chloroquinolin-4-yl oxy)-3-methoxyphenyl]-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde (8e). White solid; 83% yield; mp: 241–243 °C. FTIR ν (cm⁻¹): 1674 (C=O), 1593 (C=N and C=C), 1036. ¹H-NMR (DMSO-d₆) δ ppm 3.38 (dd, J₁ = 18.1, 5.0 Hz, 1H, H-4') 3.71 (s, 3H, OCH₃-Ar-C) 3.73 (s, 3H, OCH₃-Ar-A) 3.84 (s, 6H, OCH₃x2-Ar-A) 3.96 (dd, J₁ = 18.1, 11.8 Hz, 1H, H-4') 5.64 (dd, J₁ = 11.8, 5.0 Hz, 1H, H-5') 6.44 (dd, J₁ = 5.3 Hz, 1H, H-3) 6.91 (dd, J₁ = 8.3, 1.3 Hz, 1H, Hm') 7.09 (s, 2H, Hο') 7.18 (J₁ = 1.3 Hz, 1H, Hm) 7.30 (J₁ = 8.3 Hz, 1H, Hο') 7.67 (dd, J₂ = 9.0, 1.8 Hz, 1H, H-6) 8.07 (J₂ = 1.8 Hz, 1H, H-8) 8.34 (J₁ = 9.0 Hz, 1H, H-5) 8.68 (J₁ = 5.3 Hz, 1H, H-2) 8.97 (s, 1H, CHO) ¹³C-NMR (DMSO-d₆) δ ppm 42.5, 55.9, 56.1, 58.4, 60.2, 103.5, 104.3, 110.3, 111.3, 118.1, 118.8, 123.0, 123.8, 126.1, 126.8, 127.4, 134.8, 139.7, 140.4, 140.7, 149.5, 151.2, 152.8, 153.1, 156.1, 159.8, 160.8. MS (70 eV) m/z (%): 561 (1, M⁺), 547 (100). Anal. Calcd. For C₂₇H₂₆ClN₅O₆: C, 63.56; H, 4.78; N, 7.67. Found: C, 63.32; H, 4.48 N, 7.61.

5-[4-(7-Chloroquinolin-4-yl oxy)-3-methoxyphenyl]-3-(p-tolyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde (8f). White solid; 86% yield; mp: 233–235 °C. FTIR ν (cm⁻¹): 1668 (C=O), 1601 (C=N and C=C). ¹H-NMR (DMSO-d₆) δ ppm 2.37 (s, 3H, CH₃) Not observed (dd, J₁ = 11.7, 4.8 Hz, 1H, H-5') 6.45 (J₁ = 5.2 Hz, 1H, H-3) 6.91 (J₁ = 6.5 Hz, 1H, Hm') 7.18 (s, 1H, Hm) 7.24–7.36 (m, 3H, Hο', Hο'') 7.61 - 7.76 (m, 3H, H-6, Hm'') 8.07 (J₂ = 2.0 Hz, 1H, H-8) 8.34 (J₁ = 9.0 Hz, 1H, H-5) 8.68 (J₁ = 5.2 Hz, 1H, H-2) 8.95 (s, 1H, CHO) ¹³C-NMR (DMSO-d₆) δ ppm 21.0, 42.4, 55.9, 58.3, 103.6, 111.4, 117.8, 118.2, 122.9, 123.8, 126.7, 126.8, 127.4, 128.0, 128.6, 129.4, 132.4, 134.2, 135.6, 140.5, 149.8, 152.9, 155.6, 159.8, 160.9. MS (70 eV) m/z (%): 485 (1, M⁺), 471(100). Anal. Calcd. For C₂₇H₂₆ClN₅O₆: C, 68.71; H, 4.70; N, 8.90. Found: C, 68.66; H, 4.78; N, 8.93.

3.3. Anticancer Activity

The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO₂, 95% air and 100% relative humidity for 24 h prior to addition of 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 (T₂). 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 compounds 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. Additional four, 10-fold or ½ log serial dilutions were made to provide a total of five drug concentrations plus 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 [30]. After the tested compounds were added, the plates were incubated for an additional 48 h at 37 °C, 5% CO₂, 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. Sulfurhodamine 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 (T₀), 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: \[
\frac{(Tᵢ - T₀)}{(C - T₀)} \times 100
\] for concentrations for which \(Tᵢ > T₀\), and \[
\frac{(Tᵢ - T₀)}{T₀} \times 100
\] for concentrations for which \(Tᵢ < T₀\). Growth inhibition of 50% (GI₅₀) was calculated from \[
\frac{(Tᵢ - T₀)}{(C - T₀)} \times 100 = 50
\], 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. The drug concentration resulting in total growth inhibition (TGI) was calculated from \(Tᵢ = T₀\). The LC₅₀ (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 following treatment was calculated from \[
\frac{(Tᵢ - T₀)}{T₀} \times 100 = -50
\]. Values were calculated for each of these three 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 [30–33].

4. Conclusions

Novel series of NH 6a–f, N-acetyl 7a–f and N-formyl 8a–f pyrazoline derivates were synthesized starting from chalcones 5 bearing a quinoline motive in their structures, through an interesting synthetic methodology, affording those products in acceptable to excellent yields and in short reaction times. The antitumor evaluation data revealed that derivatives 7b,d,f and 8c,f exhibited remarkable activity with GI₅₀ values in the range from \(10^{-7}\) to \(10^{-6}\) M against different cancer cell lines. Owing to the results obtained, chemical studies are being conducted to improve the antitumor activities of such compounds.

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**Author Contributions**

The authors AM, JQ, RA, MN, JC and BI designed and accomplished research. Also, they analyzed data and wrote the paper together. Finally, all authors read and approved the final manuscript.

**Conflicts of Interest**

The authors declare no conflict of interest.

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*Sample Availability:* Samples of the compounds are available from the authors.

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