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ORIGINAL PAPER

Synthesis and biological evaluation of 3-(2,4-dichlorophenoxymethyl)-1-phenyl-1*H*-pyrazole derivatives as potential antitumor agents

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Abstract
Series of novel 3-(2,4-dichlorophenoxymethyl)-1-phenyl-1*H*-pyrazole derivatives with various substituents at positions 4 and 5 were synthesized. Antitumor activity of all the synthesized compounds was evaluated by using SRB assay on four cell lines: lung carcinoma (A549), human hepatocellular carcinoma (HepG2), colon carcinoma (HCT116) and mammary gland breast cancer (MCF-7) cell lines. Some of the synthesized compounds showed promising activity in comparison with doxorubicin against lung carcinoma cell lines. Incorporating arylidene moiety at position-4 in pyrazole ring improved and displayed strong effect on the cytotoxic activity. Compound **9c** incorporating 4-chlorophenyl moiety was the most potent toward all tested cell lines.

Keywords Pyrazoles · Schiff's bases · Hydrazones · Vinyl derivative · Antitumor agents

Introduction

Pyrazole is one of the important moieties of heterocyclic compounds. The first pyrazole was synthesized by Knorr in 1883, which led to discovery of antipyrine and its derivatives. Great attention has been focused on pyrazole derivatives as potent anti-inflammatory, analgesic, antipyretic agents [1–3]. Pyrazoles have important roles among antitumor agents; they play as good inhibitor against EGFR, $BRAF^{V600E}$, ROS receptor tyrosine kinase, telomerase, and Aurora-A kinase. There are many of drugs incorporating pyrazole moiety such as antipyrine, celecoxib, novalgine, phenylbutazone, apixaban, ramifenazone, rimonabant, fipronil, pyrazofurin [4–6]. Moreover, many of pyrazole derivatives have another broad spectra of biological activities, such as antimicrobial [7] antiviral [8] and herbicidal activities [9].

Dichlorophenoxy moiety showed systemic herbicide. (2,4-Dichlorophenoxy) acetic acid (2,4-D) are used in the control of broadleaf weeds. During the last six decades, many research were carried out on (2,4-dichlorophenoxy) acetic acid [10]. Schiff's base derivatives are interesting in medicinal chemistry due to their diverse biological properties, including antitumor activity [11]. Many articles reported that, the high electronegativity of halogens in the drug molecules plays an important role in enhancing their biological activity [12].

Treatment of cancer is a significant challenge for medicinal chemists over the past 100 years. Nevertheless, cancer is still the causing leader of death; so, there are pressing needs for novel and effective agents. Most of known chemotherapy has toxic prosperity, So, we are in need to discover novel agents that have selectively to kill tumor cells or inhibit their

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proliferation without being generally toxic as well as they are more active [13, 14].

As view in Fig. 1, we have focused on the design of novel structural entities that incorporate 1-phenyl-1*H*-pyrazole, 2,4-dichlorophenoxymethyl, azomethine (or olefin), chlorine (or pyrrolidine) into a single molecular scaffold to evaluate the potential additive anticancer effect of these systems on anticancer activity with the aim to obtain newer better-tolerated antitumor drugs and more efficacious as well as avoiding toxicity drawbacks.

Results and discussion

Chemistry

Three different types of pyrazole derivatives **1–3** were synthesized and used as starting materials for synthesizing more varieties of 4,5-disubstituted pyrazole derivatives. The first type of the aimed pyrazole derivative **1** was prepared according to the reported method [15] through treatment of ethyl 4-(2,4-dichlorophenoxy)-3-oxo-butanoate with phenylhydrazine in acetic acid. The pyrazolone derivative **1** was used as a precursor for the synthesis second type of pyrazole derivative. Thus, the pyrazolone **1** was subjected to a Vilsmeir–Haack chloroformylation and afforded product which was identified as 5-chloro-pyrazole-4-carbaldehyde derivative **2** [15]. The third type of pyrazole derivative was obtained by nucleophilic substitution reaction of **2** with pyrrolidine in DMSO, to afford pyrazole-4-carbaldehyde derivative **3** (Scheme 1). ^{1}H NMR spectrum of the aldehyde **3** displayed signals at $\delta = 1.80$, 3.09 and 3.23 ppm characteristic for the protons of pyrrolidinyl moiety as well as signal at $\delta = 9.94$ ppm characteristic for the proton of formyl group. Furthermore, its ^{13}C NMR spectrum revealed signals at $\delta = 24.2$, 25.8, 44.9 and 52.6 ppm characteristic for the carbons of pyrrolidinyl moiety as well as signal at: $\delta = 183.1$ ppm characteristic for the carbon of formyl group. The scope of the reaction of pyrazole-4-carbaldehyde **2** with various nitrogen nucleophile was studied with the objective of obtaining biologically active compounds. Thus, the reaction of pyrazole-4-carbaldehyde **2** with the selected amines in ethanol led to the formation of Schiff's bases **4a–d** in high yield. By the similar way, the scope of the reaction of pyrazole-4-carbaldehyde **3** with the same selected amines was carried out and the corresponding Schiff's bases **5a–d** were obtained in moderate yields. IR spectra of **4a–d** and **5a–d** showed disappearance of bands for carbonyl group and the presence of bands around 1614 cm^{-1} region for CH=N group. Their ^{1}H NMR spectra showed absence of aldehydic group and revealed signal for azomethine about 8.50 ppm. ^{1}H NMR spectrum of **4d**, as an example, exhibited characteristic signals for aromatic protons, beside three singlet signals for CH_3, $OCH_2$and CH=N protons at $\delta = 2.32$, 5.55 and 8.56 ppm. Moreover, its ^{13}C NMR spectrum revealed 24 carbon types, the most important signal displayed at $\delta = 21.0$ and 64.7 corresponding to CH_3 and OCH_2. For obtain biologically active compounds, the reaction of pyrazole-4-carbaldehyde derivatives **2** and **3** with isonicotinichydrazide was carried out in dioxane and led to the formation of the hydrazone derivative **6a** and **6b**, respectively, in good yields. ^{1}H NMR spectrum of hydrazone derivative **6b** revealed signal at $\delta = 11.82$ ppm (D_2O-exchangeable) for NH proton, aromatic protons were appeared in the range of 7.35–8.80 ppm, and also, aliphatic protons of pyrrolidine moiety were observed as expected positions.

Nitrovinyl derivative **7** was obtained by condensation reaction of pyrazole-4-carbaldehyde **3** with nitromethane in dioxane in the presence few drops of piperidine (Scheme 2). Its ^{1}H NMR spectrum displayed doublet signal at $\delta = 8.41$ ppm with a coupling constant indicating trans-olefinic protons. Condensation of the pyrazol-3-aldehyde derivative **3** with malononitrile afforded the corresponding vinyl derivative **8**. Its IR spectrum exhibited absorption bands at 2219 cm^{-1} for nitrile functional group. ^{1}H NMR spectrum exhibited a singlet signal at 8.41 ppm for olefinic proton. ^{13}C NMR spectrum showed two signals at 25.8 and 51.6 ppm for pyrrolidine carbons as well as signal at 66.0 ppm for methylene carbon.

Finally, aiming to reach new biologically active compounds, some arylidene derivatives were synthesized. Treatment of the pyrazolone derivative **1** with aromatic aldehydes in glacial acetic acid containing catalytic amount of sodium acetate under reflux, condensation

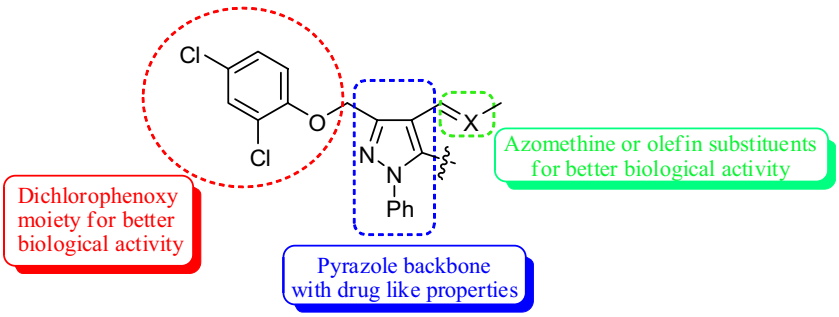
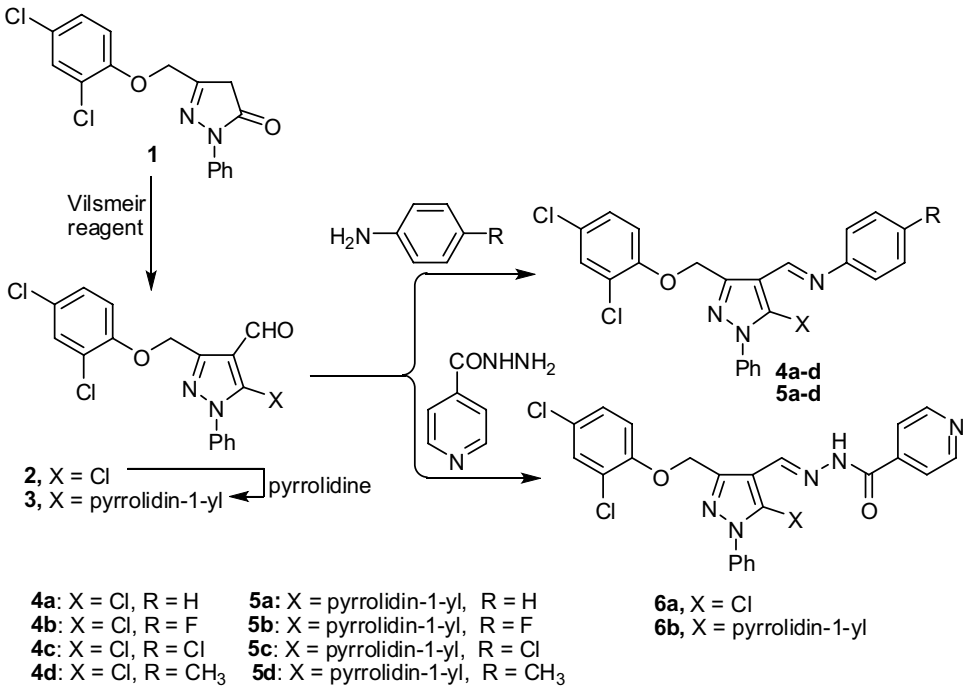
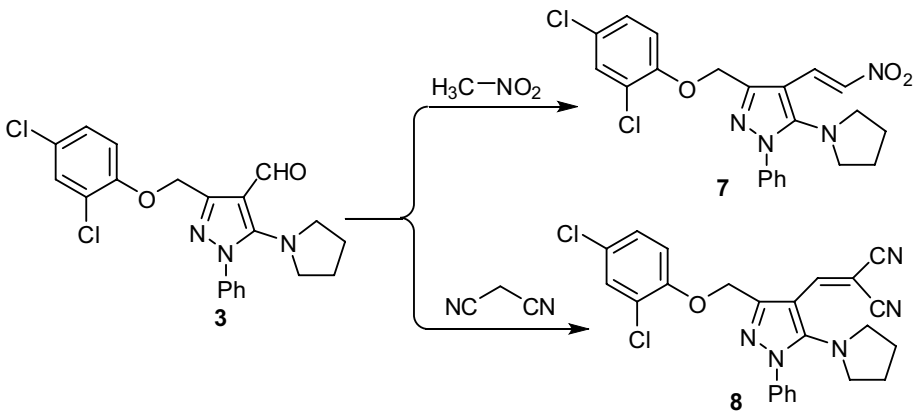


Fig. 1 Design of novel hybrid molecular scaffold

Scheme 1 Syntheses of pyrazolone **1**, formylpyrazoles **2,3**, Schiff's bases **4,5** and hydrazone derivatives **6**



Scheme 2 Syntheses of vinyl derivatives **7** and **8**



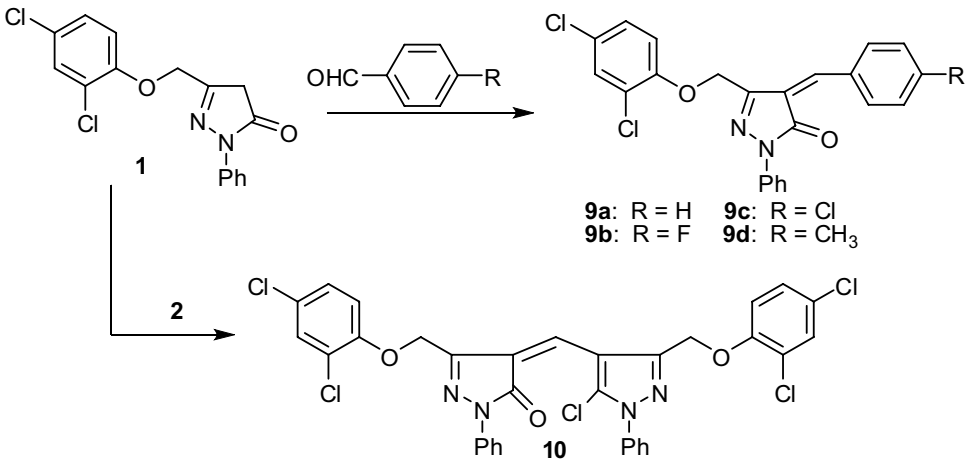
reaction was occurred and afforded the corresponding arylidene derivatives **9a–d** (Scheme 3). Moreover, condensation of pyrazolone derivative **1** with pyrazole-4-carbaldehyde **2** afforded the corresponding vinyl derivative **10**.

Anticancer activity

The aim of the present investigation was synthesizing new series of pyrazole derivatives which bearing 3-((2,4-dichlorophenoxy)methyl) at C-(3), phenyl at N-(1) and various substituent at C-(4) and others at C-(5). The antitumor activity of these derivatives was measured. The effect of each substituent at C-(4) and those at C-(5) on these activities was studied and make a comparative study between them to deduce a structure activity relationship.

Antitumor properties of the synthesized compounds in comparison with the standard drug, doxorubicin, were evaluated by using sulforhodamine B colorimetric (SRB) assay [16] on four cancer cell lines: lung carcinoma cell line (A549), human hepatocellular carcinoma cell line (HepG2), colon carcinoma cell line (HCT116) and mammary gland breast cancer cell line (MCF-7). IC_{50} values for the tested compounds are reported in Table 1. As general, all synthesized compounds exhibited good to excellent effect toward the tested cancer cell lines. The tested compounds in general

Scheme 3 Syntheses of arylidene derivatives **9a–d** and **10**



showed to be more selective toward A549 then HepG2 then HCT116 than MCF-7.

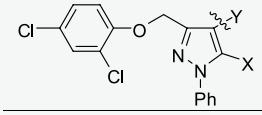
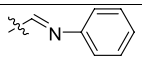
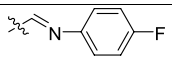
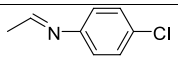
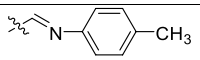
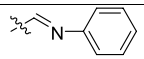
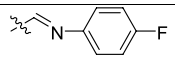
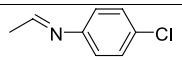
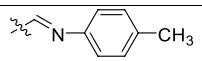
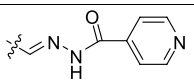
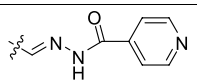
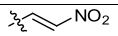
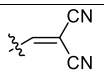
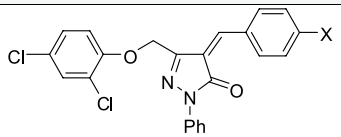
The key precursor, 5-chloro-1*H*-pyrazole-4-carbaldehyde **2** displayed weak antitumor activity. Replacing the chlorine atom in compound **2** by pyrrolidine moiety as in the key precursor **3** improved the activity compared to its analogue **2**. Structure **4** has at position-4 azomethine side chain ending with aryl moiety (aryl: phenyl, **4a**; 4-fluorophenyl, **4b**; 4-chlorophenyl, **4c**; 4-methylphenyl, **4d**). The effect of each substituent on benzene ring on the activity of the Schiff's bases **4a–d** was studied. The obtained IC_{50} values for these compounds suggest that all of the evaluated Schiff's bases (except **4c**) displayed about 50% less activity compared to doxorubicin against A549 cell line. Generally, phenyl moiety (**4a**) showed the best result toward all cell lines followed by methylphenyl moiety (**4d**). Changing the substituent on C-(5) from chlorine atom to pyrrolidine was carried out to show the difference between Cl and pyrrolidine on the effect of the biological activities. Like structure **4**, changing the substituent at C-(4) from phenyl to 4-fluorophenyl to 4-chlorophenyl to 4-methylphenyl (**5a/5b/5c/5d**) was carried out for the same reason. The obtained IC_{50} values for these compounds suggest that all of the evaluated Schiff's bases **5b** and **5c** possess significant cytotoxic activity against most of the tested cell lines used in these assays. Interestingly, unlike **4a**, its analogue **5a** showed activity less than its analogue. Pointing to the reinforcing and opposing effects of Y groups, a clear difference in cytotoxic activity is noted between compounds within this series, which may be augmented or reduced depending on whether a matching or mismatching relationship exists with other groups. So, the type of the substitutions on the benzene ring of aryl moiety is important.

Structure **6** has at position-4 isonicotinohydrazide moiety. Compounds **6a, b** showed weak activities against all tested cell lines. Replacement the substituent at position-4 from azomethine side chain moiety at structures **4** and **5** to isonicotinohydrazide moiety at structure **6** has a detrimental effect, where compounds **6a** and **6b** showed good activity against most of the tested cell lines but less than most of their analogues.

Structures **7** and **8** have at position-4 olefinic side chain bond ending with nitro group (**7**), dicyano (**8**). The presence of nitro group displayed strong effect on the cytotoxic activity and is one of the highest cytotoxic compounds among all the compounds investigated in this study, where **7** showed results near to the reference drug against all tested cell lines. The presence of dicyano has a detrimental effect where compound **8** displayed weak effects on the cytotoxic activity. For searching for newer antitumor, series of arylidene derivatives **9a–d** were synthesized. Arylidene derivatives **9a–d** has at position-4 olefinic side chain bond ending with aryl moiety (aryl: phenyl, **9a**; 4-fluorophenyl, **9b**; 4-chlorophenyl, **9c**; 4-methylphenyl, **9d**). The obtained IC_{50} values for these compounds suggest that all of the evaluated arylidenes **9a–d** possess significant cytotoxic activity against most of the tested cell lines used in these assays. In general, incorporating arylidene moiety at position-4 improved the cytotoxic activity. Thus, arylidene moiety displayed strong effect on the cytotoxic activity. The latter series is the highest cytotoxic compounds among all the series investigated in this study, where **9b–d** showed potency near todoxorubicin in inhibiting the growth of most of cell lines.

Increasing the size of the substituent as in structure **10** had a good effect on antitumor activity. Structure **10**, which has olefinic bond ending with pyrazole moiety showed good activity against all tested cell lines (except MCF-7). Comparison between the anticancer activity of our potent synthesized compounds and doxorubicin as standard anticancer reference drug against the used cell lines is represented graphically in Fig. 2.

Table 1 Antitumor of all compounds and doxorubicin against the tested cancerous cell lines

Compound no.			IC_{50} (μM/L) ± SE			
	X	Y	A549	HEPG-2	HCT 116	MCF-7
2	Cl		347.9 ± 3.7	291.9 ± 3.0	325.3 ± 3.9	464.0 ± 4.5
3	Pyrrolidin-1-yl		111.1 ± 2.6	125.8 ± 2.5	150.9 ± 2.45	208.6 ± 2.5
4a	Cl		10.6 ± 1.0	13.0 ± 0.7	29.0 ± 0.6	65.0 ± 2.3
4b	Cl		9.8 ± 0.8	50.8 ± 1.7	42.4 ± 1.7	69.0 ± 2.1
4c	Cl		59.0 ± 2.1	22.6 ± 0.8	46.9 ± 1.7	63.5 ± 2.2
4d	Cl		11.46 ± 1.0	20.1 ± 1.1	49.1 ± 1.7	60.4 ± 2.1
5a	Pyrrolidin-1-yl		109.8 ± 2.8	117.1 ± 2.8	127.6 ± 2.5	151.5 ± 3.2
5b	Pyrrolidin-1-yl		10.7 ± 0.8	13.3 ± 0.7	17.3 ± 0.7	23.3 ± 1.4
5c	Pyrrolidin-1-yl		34.0 ± 0.6	20.2 ± 1.5	24.7 ± 0.8	42.2 ± 1.7
5d	Pyrrolidin-1-yl		83.2 ± 2.1	74.6 ± 0.6	84.8 ± 2.0	110.9 ± 2.5
6a	Cl		42.0 ± 1.6	63.5 ± 1.8	47.2 ± 1.7	59.6 ± 2.1
6b	Pyrrolidin-1-yl		36.0 ± 0.6	40.2 ± 1.8	43.6 ± 1.7	61.5 ± 2.2
7	Pyrrolidin-1-yl		7.6 ± 0.1	7.3 ± 0.1	5.2 ± 0.5	12.0 ± 0.9
8	Pyrrolidin-1-yl		86.5 ± 2.0	77.7 ± 2.3	90.2 ± 2.3	123.3 ± 2.5
						
9a	H		13.7 ± 0.7	18.7 ± 1.1	11.2 ± 0.9	12.8 ± 1.1
9b	F		5.5 ± 0.5	10.1 ± 1.0	7.8 ± 0.6	14.7 ± 0.7
9c	Cl		5.9 ± 0.6	7.9 ± 0.6	7.1 ± 0.5	7.4 ± 0.6
9d	CH_3		5.2 ± 0.5	7.0 ± 0.5	11.7 ± 1.0	14.3 ± 0.9
10			16.1 ± 0.7	17.9 ± 1.1	19.3 ± 1.1	40.0 ± 1.0
Doxorubicin			5.53 ± 0.56	7.84 ± 0.26	8.29 ± 0.84	7.56± 0.13

Conclusions

In general, it can be concluded that treatment of pyrazole-4-carbaldehyde derivative with active methylene and nitromethane improved the antitumor properties against the tested cell lines. Also, treatment of the pyrazolone derivative **1** with aromatic aldehydes improved the antitumor properties against the tested cell lines. The obtained IC_{50} values for these compounds suggest that incorporation at position-4 olefinic side chain bond ending with nitro group as in compound **7** showed the highest cytotoxic activity among all the investigated compounds. Incorporating arylidene moiety at position-4 as in compounds **9b, c** improved the cytotoxic activity. Thus, arylidene moiety showed potency near to doxorubicin in inhibiting the growth of the cell lines.

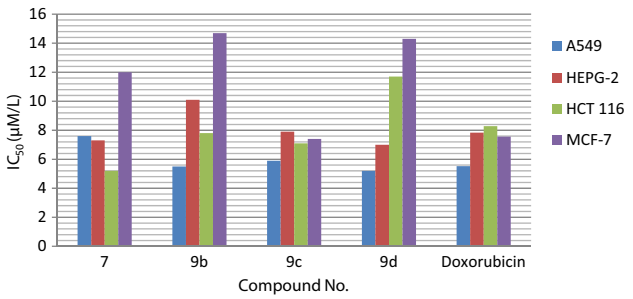


Fig. 2 Comparison between the antitumor activity of our potent synthesized compounds and doxorubicin

Experimental section

All melting points are recorded on digital Gallen Kamp MFB-595 instrument and may be uncorrected. The IR spectra (KBr) (cm^{-1}) were measured on a JASCO spectrophotometer. ^{1}H NMR spectra were recorded on Bruker spectrometers (at 500 MHz) and are reported relative to deuterated solvent signals in deuterated dimethylsulfoxide (DMSO-d_6).^{13}C NMR spectra were recorded on Bruker Spectrometers (at 125 MHz) in deuterated dimethylsulfoxide (DMSO-d_6).

Synthesis of 3-((2,4-dichlorophenoxy) methyl)-1-phenyl-5-(pyrrolidin-1-yl)-1*H*-pyrazole-4-carbaldehyde (3)

To a solution of **2** (0.01 mol) in 25 mL DMSO, pyrrolidine (0.01 mol) was added and the reaction mixture was stirred for 24 h at room temperature. The solution was diluted with H_2O (100 mL) and extracted with Et_2O (3 × 50 mL). The organic phase was washed with H_2O (100 mL), after which it was dried (Na_2SO_4). Removal of the solvent left a solid residue, which was purified by column chromatography (ethyl acetate: *n*-hexane\4:1). Yield 75%; m.p. 138–140 °C; IR: ν/cm^{-1} = 3065 (CH–Ar.), 2952, 2923, 2883 (CH-aliph.), 1603 (C=N); ^{1}H NMR: δ/ppm = 1.80 (s, 4H, 2CH_2-pyrrolidinyl), 3.23 (s, 2H, CH_2-pyrrolidinyl), 5.28 (s, 2H, OCH_2), 7.31 (d, 2H, *J* = 8.92 Hz, Ar–H), 7.39 (d, 2H, *J* = 8.70 Hz, Ar–H), 7.44–7.54 (m, 3H, Ar–H), 7.57 (s, 1H, Ar–H), 9.94 (br, 1H, CHO); ^{13}C NMR: 24.2 (CH_2), 25.8 (CH_2), 44.9 (CH_2), 52.6 (CH_2), 65.4 (OCH_2), 108.8 (C), 115.9 (CH), 122.8 (C), 125.0 (C), 126.8 (2CH), 128.5 (CH), 129.0 (CH), 129.5 (2CH), 129.7 (CH), 140.2 (C), 149.0 (C), 151.5 (C), 153.5 (CH), 183.1 (CHO); MS, m/z (%): 416 (M^+; 55.6); Anal. Calcd for $C_{21}H_{19}Cl_2N_3O_2$ (416.30): C, 60.59; H, 4.60; N, 10.09; Found: C, 60.72; H, 4.57; N, 9.87%.

Synthesis of Schiff's bases derivatives 4a–d and 5a–d

A solution of the aldehyde **2** or **3** (0.01 mol) in 50 mL ethanol was treated with the requisite amine (0.01 mol) (namely aniline, 4-fluoroaniline, 4-chloroaniline or 4-methylaniline). The reaction mixture was heated under reflux for 3 h. The solution was left to cool. The solid product obtained was filtered off and crystallized from THF to give the final products.

N-((5-Chloro-3-((2,4-dichlorophenoxy) methyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)aniline (4a)

Yield 85%; m.p. 126–128 °C; IR: ν/cm^{-1} = 3061 (CH–Ar.), 2941, 2866 (CH-aliph.), 1626 (C=N); ^{1}H NMR: δ/ppm = 5.55 (s, 2H, OCH_2), 7.1 (d, 1H, *J* = 7.70 Hz, Ar–H), 7.19–7.30 (m, 1H, Ar–H), 7.38–7.50 (m, 5H, Ar–H), 7.56–7.68 (m, 5H, Ar–H), 8.02 (d, 1H, *J* = 8.10 Hz, Ar–H), 8.56 (s, 1H, CH=N); ^{13}C NMR: 64.7 (OCH_2), 116.2 (CH), 118.5 (CH), 121.2 (2CH), 123.0 (C), 125.3 (C), 125.9 (2CH), 126.5 (CH), 128.5 (CH), 129.4 (CH), 129.7 (2CH), 129.8 (CH), 129.9 (2CH), 130.2 (CH), 137.4 (C), 148.4 (C), 151.1 (CH=N); 152.0 (C), 153.4 (C); MS, m/z (%): 456 (M^+; 48.2); Anal. Calcd for $C_{23}H_{16}Cl_3N_3O$ (456.75): C, 60.48; H, 3.53; N, 9.20; Found: C, 60.56; H, 3.42; N, 9.13%.

N-((5-Chloro-3-((2,4-dichlorophenoxy) methyl)-1-phenyl-1*H*-pyrazol-4-yl) methylene)-4-fluoroaniline (4b)

Yield 81%; m.p. 120–122 °C; IR: ν/cm^{-1} = 3071 (CH–Ar.), 2923 (CH-aliph.), 1629 (C=N); ^{1}H NMR: δ/ppm = 5.54 (s, 2H, OCH_2), 7.21–7.25 (m, 4H, Ar–H), 7.39- 7.47 (m, 3H, Ar–H), 7.58–7.68 (m, 5H, Ar–H), 8.57 (s, 1H, CH=N); ^{13}C NMR: 64.7 (OCH_2), 116.1 (CH), 116.3 (d, $J_{C\text{-}F}$ = 22.3 Hz, CH), 123.0 (CH), 123.1 (CH), 125.3 (C), 125.9 (CH), 128.5 (CH), 129.8 (d, $J_{C\text{-}F}$ = 9.3 Hz, CH), 129.9 (CH), 130.0 (CH), 130.1 (CH), 130.3 (C), 137.3 (C), 148.4 (d, $J_{C\text{-}F}$ = 2.7 Hz, C–N), 151.2 (CH=N), 153.4 (C), 161.03 (d, $J_{C\text{-}F}$ = 242.7 Hz, C-F); MS, m/z (%): 474 (M^+; 39.6); Anal. Calcd for $C_{23}H_{15}Cl_3FN_3O$ (474.74): C, 58.19; H, 3.18; N, 8.85; Found: C, 58.23; H, 3.07; N, 8.76%.

4-Chloro-*N*-((5-chloro-3-((2,4-dichlorophenoxy) methyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)aniline (4c)

Yield 85%; m.p. 116–118 °C; IR: ν/cm^{-1} = 3072 (CH–Ar.), 2921, 2851 (CH-aliph.), 1627 (C=N); ^{1}H NMR: δ/ppm = 5.55 (s, 2H, OCH_2), 7.19 (d, 2H, *J* = 8.6 Hz, Ar–H), 7.39–7.46 (m, 4H, Ar–H), 7.56–7.68 (m, 6H, Ar–H), 8.58

(s, 1H, CH=N); ^{13}C NMR: 64.7 (OCH_2), 116.1 (CH), 116.3 (CH), 123.0 (CH), 123.1 (2CH), 125.3 (C), 125.9 (2CH), 128.6 (CH), 129.6 (2CH), 129.8 (CH), 129.9 (CH), 130.0 (2CH), 130.5 (C), 130.8 (C), 137.3 (C), 148.5 (C), 150.8 (C), 151.9 (CH=N), 153.4 (C); MS, m/z (%): 491 (M^+; 82.1); Anal. Calcd for $C_{23}H_{15}Cl_4N_3O$ (491.19): C, 56.24; H, 3.08; N, 8.55; Found: C, 56.37; H, 2.99; N, 8.43%.

N-((5-Chloro-3-((2,4-dichlorophenoxy) methyl)-1-phenyl-1*H*-pyrazol-4-yl) methylene)-4-methylaniline (4d)

Yield 81%; m.p. 145–147 °C; IR: $\nu/cm^{-1} = 3022$ (CH–Ar.), 2920 (CH-aliph.), 1627 (C=N); ^{1}H NMR: δ/ppm = 2.32 (s, 3H, CH_3), 5.55 (s, 2H, OCH_2), 7.06 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.20 (d, 2H, $J = 7.8$ Hz, Ar–H), 7.41 (s, 2H, Ar–H), 7.76–7.50 (m, 6H, Ar–H), 8.56 (s, 1H, CH=N); ^{13}C NMR: 21.0 (CH_3), 64.7 (OCH_2), 116.1 (CH), 116.3 (CH), 121.2 (2CH), 123.0 (C), 125.3 (C), 125.9 (2CH), 128.6 (CH), 129.6 (CH), 129.8 (CH), 129.9 (2CH), 130.0 (2CH), 130.5 (C), 130.8 (C), 137.3 (C), 148.5 (C), 150.8 (C), 151.9 (CH=N), 153.4 (C); MS, m/z (%): 470 (M^+; 72.2); Anal. Calcd for $C_{24}H_{18}Cl_3N_3O$ (470.78): C, 61.23; H, 3.85; N, 8.93; Found: C, 61.42; H, 3.74; N, 8.72%.

N-((3-((2,4-Dichlorophenoxy)methyl)-1-phenyl-5-(pyrrolidin-1-yl)-1*H*-pyrazol-4-yl)methylene) aniline (5a)

Yield 76%; m.p. 144–146 °C; IR: $\nu/cm^{-1} = 3057$ (CH–Ar.), 2975, 2927, 2875 (CH-aliph.), 1634 (C=N); ^{1}H NMR: δ/ppm = 1.80 (s, 4H, pyrrolidinyl-H), 3.27 (s, 4H, pyrrolidinyl-H), 5.41 (s, 2H, OCH_2), 7.00 7.22 (m, 5H, Ar–H), 7.34–7.73 (m, 8H, Ar–H), 8.68 (s, 1H, CH=N); ^{13}C NMR: 25.7 (CH_2), 51.7 (CH_2), 65.8 (OCH_2), 107.7 (CH), 116.1 (CH), 116.3, 122.0, 122.4, 123.1, 125.0, 125.6 (2CH), 128.4, 129.2 (2CH), 129.4, 140.0, 147.7, 150.1 (CH=N), 153.8; MS, m/z (%): 491 (M^+; 63.2); Anal. Calcd for $C_{27}H_{24}Cl_2N_4O$ (491.4): C, 65.99; H, 4.92; N, 11.40; Found: C, 66.12; H, 4.89; N, 11.34%.

N-((3-((2,4-Dichlorophenoxy)methyl)-1-phenyl-5-(pyrrolidin-1-yl)-1*H*-pyrazol-4-yl) methylene)-4-fluoroaniline(5b)

Yield 73%; m.p. 158–160 °C; IR: $\nu/cm^{-1} = 3064$ (CH–Ar.), 2954, 2924, 2884 (CH-aliph.), 1607 (C=N); ^{1}H NMR: δ/ppm = 1.82 (s, 4H, pyrrolidinyl-H), 3.26 (s, 4H, pyrrolidinyl-H), 5.43 (s, 2H, OCH_2), 7.09 (dd, 2H, $J = 8.7, 5.2$ Hz, Ar–H), 7.17 (t, 2H, $J = 8.7$ Hz, Ar–H), 7.34–7.43 (m, 2H, Ar–H), 7.61–7.83 (m, 6H, Ar–H), 8.68 (s, 1H, CH = N); ^{13}C NMR: 25.8 (CH_2), 51.8 (CH_2), 65.7 (OCH_2), 107.5 (CH), 116.0 (d, $J_{C\text{-}F} = 22.3$ Hz, CH), 116.2, 122.6, 122.9, 124.9, 125.7 (CH), 128.5, 129.6 (d, $J_{C\text{-}F} = 8.9$ Hz, CH), 129.7, 140.2, 5 149.5 (d, $J_{C\text{-}F} = 2.7$ Hz, C–N), 150.0 (CH=N), 153.7, 160.3 (d, $J_{C\text{-}F} = 240.9$ Hz, C-F); MS, m/z (%): 509 (M^+; 55.2); Anal. Calcd for $C_{27}H_{23}Cl_2FN_4O$ (509.40): C, 63.66; H, 4.55; N, 11.00; C, 64.25; H, 4.81; N, 10.70; Found: C, 63.73; H, 4.42; N, 10.87%.

4-Chloro-*N*-((3-((2,4-dichlorophenoxy) methyl)-1-phenyl-5-(pyrrolidin-1-yl)-1*H*-pyrazol-4-yl)methylene)aniline (5c)

Yield 75%; m.p. 146–148 °C; IR: $\nu/cm^{-1} = 3065$ (CH–Ar.), 2952, 2923, 2883 (CH-aliph.), 1603 (C=N); ^{1}H NMR: δ/ppm = 1.83 (s, 4H, pyrrolidinyl-H), 3.27 (s, 4H, pyrrolidinyl-H), 5.41 (s, 2H, OCH_2), 7.09 - 7.17 (m, 4H, Ar–H), 7.30–7.80 (m, 8H, Ar–H), 8.70 (s, 1H, CH=N); ^{13}C NMR: δ = 25.4 (CH_2), 51.5 (CH_2), 65.2 (OCH_2), 107.4 (CH), 116.1 (CH), 116.5, 122.2, 122.4, 122.5, 124.7, 125.6 (2CH), 128.7, 129.2 (2CH), 129.4, 140.0, 148.1, 149.5, 153.1; MS, m/z (%): 525 (M^+; 33.6); Anal. Calcd for $C_{27}H_{23}Cl_3N4O$ (525.9): C, 61.67; H, 4.41; N, 10.65; Found: C, 61.45; H, 4.39; N, 10.54%.

N-((3-((2,4-dichlorophenoxy)methyl)-1-phenyl-5-(pyrrolidin-1-yl)-1*H*-pyrazol-4-yl) methylene)-4-methylaniline (5d)

Yield 70%; m.p. 140–142 °C; IR: $\nu/cm^{-1} = 3065$ (CH–Ar.), 2952, 2923, 2883 (CH-aliph.), 1603 (C=N); ^{1}H NMR: δ/ppm = 1.81 (s, 4H, $2CH_2$), 2.21 (s, 3H, CH_3), 3.27 (s, 4H, $2CH_2$), 5.41 (s, 2H, OCH_2), 7.00–7.80 (m, 8H, Ar–H), 8.63 (s, 1H, CH=N); ^{13}C NMR: 20.2 (CH_3), 25.4 (CH_2), 51.5 (CH_2), 65.2 (OCH_2), 107.4, 116.1, 116.5,122.2, 122.4, 122.5, 124.7, 125.6, 126.4, 128.7, 129.2, 129.3, 129.4, 140.0, 148.1, 149.3, 153.0; MS, m/z (%): 505 (M^+; 45.6); Anal. Calcd for $C_{28}H_{26}Cl_2N_4O$ (505.4): C, 66.54; H, 5.18; N, 11.08; Found: C, 66.62; H, 5.23; N, 10.98%.

Synthesis of hydrazide derivatives 6a and 6b

A mixture of aldehyde **1** or **2** (0.01 mol) and isonicotinichydrazide (0.01 mol) in dioxane (30 mL) was heated under reflux for 1 h. The reaction mixture was concentrated under vacuum, left to cool. The solid product obtained was filtered off and crystallized from ethanol to give the final products.

N'-((5-chloro-3-((2,4-dichlorophenoxy) methyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)isonicotinohydrazide (6a)

Yield 80%; m.p. 199–201 °C; IR: $\nu/cm^{-1} = 3065$ (CH–Ar.), 2952, 2923, 2883 (CH-aliph.), 1603 (C=N); ^{1}H NMR: δ/ppm = 5.51 (s, 2H, OCH_2), 7.35- 8.03 (m, 10H, Ar–H), 8.57

(s, 1H, CH=N), 8.81 (m, 2H, Ar–H), 12.12 (br, 1H, NH); ^{13}C NMR: 64.7, 100.4, 116.1, 116.2, 118.5, 121.1, 121.2 (2C), 123.0, 125.5, 125.9 (2C), 126.5, 128.5, 129.4, 129.7 (2C), 130.2, 130.3, 137.4, 148.4, 151.1, 152.0, 153.4; MS, m/z (%): 500 (M^+; 34.5); Anal. Calcd for $C_{23}H_{16}Cl_3N_5O_2$ (500.76): C, 55.16; H, 3.22; N, 13.99; Found: C, 55.23; H, 3.17; N, 13.87%.

N′-((3-((2,4-dichlorophenoxy)methyl)-1-phenyl-5-(pyrrolidin-1-yl)-1*H*-pyrazol-4-yl)methylene) isonicotinohydrazide (6b)

Yield 72%; m.p. 124–126 °C; IR: $\nu/cm^{-1} = 3444$, 3205 (NH), 3046 (CH–Ar.), 2975, 2952, 2874 (CH-aliph.), 1666 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ/ppm = 1.88 (s, 4H, pyrrolidinyl-H), 3.20 (s, 4H, pyrrolidinyl-H), 5.39 (s, 2H, OCH_2), 7.35–7.71 (m, 9H, Ar–H), 7.82 (s, 1H, Ar–H), 8.68 (s, 1H, CH=N), 8.80 (s, 2H, Ar–H), 11.82 (br, 1H, NH); ^{13}C NMR (126 MHz,, $CDCl_3$): δ = 25.8, 51.8, 65.7, 25.9, 51.5, 64.7, 101.8, 116.6, 121.9,125.1,125.8, 128.6,129.6, 129.7, 143.1, 146.9, 150.8, 161.3; MS, m/z (%): 535 (M^+; 43.6); Anal. Calcd for $C_{27}H_{24}Cl_2N_6O_2$ (535.4): C, 60.57; H, 4.52; N, 15.70; Found: C, 60.38; H, 4.44; N, 15.47%.

Synthesis of 3-((2,4-dichlorophenoxy)methyl)-4-(2-nitrovinyl)-1-phenyl-5-(pyrrolidin-1-yl)-1*H*-pyrazole (7)

To equimolar amount of the aldehyde derivative **3** (0.01 mol) and nitromethane (0.01 mol) in dioxane (30 mL), few drops of piperidine was added. The reaction mixture was heated under reflux for 2 h., then allowed to cool. The obtained product was collected by filtration and crystallized from ethanol. Yield 67%; m.p. 133–135 °C; IR: $\nu/cm^{-1} = 3076$ (CH–Ar.), 2938, 2884, 2846 (CH-aliph.), 1606 (C=N); ^{1}H NMR: δ/ppm = 1.82 (m, 4H, $2CH_2$), 3.26 (s, 4H, $2CH_2$), 5.28 (s, 2H, OCH_2), 7.46 (d, 1H, Ar–H), 7.47–7.58 (m, 7H, Ar–H), 7.65 (s, 1H, Ar–H), 8.41 (d, 1H, J = 13.0 Hz, olefinic CH); ^{13}C NMR: 25.8 ($2CH_2$), 52.0 ($2CH_2$), 64.9 (OCH_2), 100.1, 116.2, 123.0, 125.7, 126.5 (2CH), 128.6, 129.1, 129.6 (2CH), 130.0, 132.0 (2C), 140.0, 146.6, 151.3, 152.4; MS, m/z (%): 459 (M^+; 49.3); Anal. Calcd for $C_{22}H_{20}Cl_2N_4O_3$ (459.33): C, 57.53; H, 4.39; N, 12.20; Found: C, 57.42; H, 4.42; N, 12.08%.

Synthesis of2-((3-((2,4-dichlorophenoxy)methyl)-1-phenyl-5-(pyrrolidin-1-yl)-1*H*-pyrazol-4-yl)methylene)malononitrile (8)

To equimolar amount of the aldehyde derivative **3** (0.01 mol) and malononitrile (0.01 mol) in dioxane (30 mL), few drops of piperidinewas added. The reaction mixture was heated under reflux for 1 h. then allowed to cool, the obtained product was collected by filtration and crystallized from dioxane. Yield 85%; m.p. 152–154 °C; IR: $\nu/cm^{-1} = 2984$, 2945, 2833 (CH-aliph.), 2219 (C≡N); ^{1}H NMR: δ/ppm = 1.82 (s, 4H, pyrrolidinyl-H), 3.17 (s, 4H, pyrrolidinyl-H), 5.34 (s, 2H, OCH_2), 7.32 (d, 1H, J = 6.1 Hz, Ar–H), 7.41 (dd, 1H, J = 6.2 Hz, Ar–H), 7.50–7.59 (m, 5H, Ar–H), 7.64 (s, 1H, Ar–H), 8.41 (s, 1H, CH-olefinic); ^{13}C NMR: 25.8 ($2CH_2$), 51.6 ($2CH_2$), 66.0 (OCH_2), 75.6 (C), 102.8 (2C≡N), 115.1, 115.9, 116.1, 122.9, 125.5, 126.6 (2CH), 128.6, 129.4, 129.7 (2CH), 139.8, 147.3, 150.8, 152.6, 153.4; MS, m/z (%): 464 (M^+; 46.6); Anal. Calcd for $C_{24}H_{19}Cl_2N_5O$ (464.35): C, 62.08; H, 4.12; N, 15.08; Found: C, 62.17; H, 4.04; N, 15.23%.

Reaction of pyrazol-5(4*H*)-one 1 with aldehydes

Solution of **1** (0.01 mol) in glacial acetic acid (20 mL) was treated with the requisite aldehydes (0.01 mL) and fused sodium acetate (0.5 gm). The reaction mixture was heated under reflux for 2 h, poured into crashed ice. The obtained product was filtered and crystallized from ethanol.

4-Benzylidene-3-((2,4-dichlorophenoxy)methyl)-1-phenyl-1*H*-pyrazol-5(4*H*)-one (9a)

Yield 77%; IR: $\nu/cm^{-1} = 1650$ (C=O); ^{1}H NMR: δ/ppm = 5.53 (s, 2H, OCH_2), 7.02–7.50 (m, 8H, Ar–H), 7.60–7.70 (m, 5H, Ar–H), 8.11 (d, 1H, J = 8.10 Hz, Ar–H); ^{13}C NMR: 64.7, 116.2, 118.5, 121.2, 123.0, 125.3, 125.9, 126.5, 128.5, 129.4, 129.7, 129.1, 129.9, 130.2, 137.4, 148.4, 151.1, 152.0, 153.4, 166.3; MS, m/z (%): 423 (M^+; 62.0); Anal. Calcd for $C_{23}H_{16}Cl_2N_2O_2$ (423.29): C, 65.26; H, 3.81; N, 6.62; Found: C, 65.41; H, 3.78; N, 6.57%.

3-((2,4-Dichlorophenoxy)methyl)-4-(4-fluorobenzylidene)-1-phenyl-1*H*-pyrazol-5(4*H*)-one (9b)

Yield 81%; IR: $\nu/cm^{-1} = 1651$ (C=O); ^{1}H NMR: δ/ppm = 5.51 (s, 2H, OCH_2), 7.20–7.50 (m, 6H, Ar–H), 7.58–7.70 (m, 7H, Ar–H); ^{13}C NMR: 64.7, 116.1, 116.2, 116.4, 123.0, 123.1, 125.3, 125.9, 128.5, 129.8, 129.9, 130.0, 130.1, 130.3, 137.3, 148.4, 151.2, 166.4; MS, m/z (%): 441 (M^+; 37.5); Anal. Calcd for $C_{23}H_{15}Cl_2FN_2O_2$ (441.28): C, 62.60; H, 3.43; N, 6.35; Found: C, 62.54; H, 3.41; N, 6.41%.

4-(4-Chlorobenzylidene)-3-((2,4-dichlorophenoxy)methyl)-1-phenyl-1*H*-pyrazol-5(4*H*)-one (9c)

Yield 83%; IR: $\nu/cm^{-1} = 1648$ (C=O); ^{1}H NMR: δ/ppm = 5.52 (s, 2H, OCH_2), 7.19–7.46 (m, 6H, Ar–H), 7.56–8.84 (m, 7H, Ar–H);^{13}C NMR: 64.7, 116.1, 116.3, 123.0, 123.1, 125.3, 125.9, 128.6, 129.6, 129.8, 129.9, 130.0,

130.5, 130.8, 137.3, 148.5, 150.8, 151.9, 153.4, 166.5; MS, m/z (%): 457 (M^+; 55.6); Anal. Calcd for $C_{23}H_{15}Cl_3N_2O_2$ (457.74): C, 60.35; H, 3.30; N, 6.12; Found: C, 60.44; H, 3.28; N, 6.04%.

3-((2,4-Dichlorophenoxy)methyl)-4-(4-methylbenzylidene)-1-phenyl-1*H*-pyrazol-5(4*H*)-one (9d)

Yield 72%; IR: $\nu/cm^{-1} = 1652$ (C=O); ^1H NMR: δ/ppm = 2.31 (s, 3H, CH_3), 5.52 (s, 2H, OCH_2), 7.06–7.50 (m, 13H, Ar–H); ^{13}C NMR: 21.0, 64.7, 116.1, 116.3, 121.2, 123.0, 125.3, 125.9, 128.6, 129.6, 129.8, 129.9, 130.0, 130.5, 130.8, 137.3, 148.5, 150.8, 153.4, 165.9; MS, m/z (%): 437 (M^+; 29.8); Anal. Calcd for $C_{24}H_{18}Cl_2N_2O_2$ (437.32): C, 65.91; H, 4.15; N, 6.41; Found: C, 66.02; H, 4.11; N, 6.34%.

Synthesis of 4-((5-chloro-3-((2,4-dichlorophenoxy)methyl)-1-phenyl-1*H*-pyrazol-4-yl) methylene)-3-((2,4-dichlorophenoxy)methyl)-1-phenyl-1*H*-pyrazol-5(4*H*)-one (10)

Solution of **1** (0.01 mol) in glacial acetic acid (20 mL) was treated with the pyrazole-4-carbaldehyde **2** (0.01 mL) and fused sodium acetate (0.5 g). The reaction mixture was heated under reflux for 2 h, poured into crashed ice. The obtained product was filtered and crystallized from DMF, Yield 68%; IR: $\nu/cm^{-1} = 1656$ (C=O); ^1H NMR: δ/ppm = 5.34 (s, 4H, $2OCH_2$), 7.20–8.30 (m, 17H, Ar–H); MS, m/z (%): 698 (M^+; 14.6); Anal. Calcd for $C_{33}H_{21}Cl_5N_4O_3$ (698.81): C, 56.72; H, 3.03; N, 8.02; Found: C, 56.83; H, 2.98; N, 7.89%.

References

1. A.A. Farag, M.F. El Shehry, S.Y. Abbas, S.N. Abd-Alrahman, A.A. Atrees, H.Z. Al-basheer, Y.A. Ammar, Z. Naturforsch. B **70**, 519–526 (2015)
2. M.F. Khan, M.M. Alam, G. Verma, W. Akhtar, M. Akhter, M. Shaquiquzzaman, Eur. J. Med. Chem. **120**, 170–201 (2016)
3. S. Ganguly, S.K. Jacob, Mini Rev. Med. Chem. **17**, 959–983 (2017)
4. S. Küçükgüzel, S. Senkardes, Eur. J. Med. Chem. **97**, 786–815 (2015)
5. I. Desai, K. Sinha, W. Null, M.A. Wolter, T. Suckow, S. King, R. Amin, Sinha. Int. J. Cancer **127**, 230–238 (2010)
6. P. Sooriakumaran, S.E. Langley, R.W. Laing, H.M. Coley, J. Chemother. **19**, 21–32 (2007)
7. M.F. El Shehry, M.M. Ghorab, S.Y. Abbas, E.A. Fayed, S.A. Shedid, Y.A. Ammar, Eur. J. Med. Chem. **143**, 1463–1473 (2018)
8. E.A.E. El-Helw, M.M. Gado, A.K. El-Ziaty, J. Iran. Chem. Soc. **17**, 1479–1492 (2020)
9. J.C. Jung, E.B. Watkins, M.A. Avery, Tetrahedron **58**, 3639–3646 (2002)
10. J. Pernak, A. Syguda, K. Materna, E. Janus, P. Kardasz, T. Praczyk, Tetrahedron **68**, 4267–4273 (2012)
11. S.Y. Abbas, K.A.M. El-Bayouki, W.M. Basyouni, E.A. Mostafa, Med. Chem. Res. **27**, 571–582 (2018)
12. S.Y. Abbas, A.A. Farag, Y.A. Ammar, A.A. Atrees, A.F. Mohamed, A.A. El-Henawy, Monatsh. Chem. **144**, 1725–1733 (2013)
13. R.A.K. Al-Harbi, M.A.M.S. El-Sharief, S.Y. Abbas, Bioorg. Chem. **90**, 103088 (2019)
14. S.Y. Abbas, R.A.K. Al-Harbib, M.A.M.S. El-Sharief, Eur. J. Med. Chem. **198**, 112363 (2020)
15. E.M. El-Telbani, M.F. El Shehry, G.A.M. Nawwar, Monatsh. Chem. **139**, 685–689 (2008)
16. P. Skehan, R. Storeng, D. Scudiero, A. Monks, M.J. Mahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenny, M.R. Boyd, J. Nat. Cancer Inst. **82**, 1107–1112 (1990)