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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of novel 4,5-dihydropyrazole derivatives as potent anticancer and antimicrobial agents

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Abstract A focused library of 4,5-dihydropyrazole derivatives (**4**, **5**, **6**, **7a–h**, **8**, **9a–g**, and **10a–g**) were synthesized from novel 5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazole-1-carbothioamide **4**. The synthesized compounds were characterized using elemental analysis and spectral data (IR, mass spectra, ^{1}H and ^{13}C NMR) and evaluated for antimicrobial activity by broth dilution method and in vitro anticancer activity. Among the synthesized compounds **7a**, **9c**, **9g**, and **10d** exhibit broad spectrum antimicrobial activity against tested microbial strains. The in vitro cancer results ascertain **7a**, **9c**, and **10d** are most potent molecules in comparison to reference standard cisplatin.

Keywords 4,5-Dihydropyrazole · Anticancer · Antimicrobial · Claisen–Schmidt reaction

Introduction

Cancer is the worldwide health problem and the most frightening disease of human (Zhang, 2002). Chemotherapy, either alone or as an adjunct to radiotherapy or surgery remains the treatment of choice in most of the cancers (Buolamwini, 1999; MacDonald, 2009).

The current anticancer agents are mostly broad acting cytotoxic drugs. They impact structure and function of the rapidly proliferating cancer cells and arrest the cell cycle at a specific phase depending on the mechanism of action of the agents (Li *et al.*, 2001; Engel *et al.*, 2003). Due to their lack in specificity and adverse effects related to impact on rapidly dividing non-cancerous cells, there is an urgent need for identification of novel, potent, selective, and less toxic agents, which can overcome cancer resistance to drug treatment that has made many of the currently available chemotherapeutic agents ineffective (Borowski *et al.*, 2005).

The α,β-unsaturated ketones (chalcones) are considered to be precursors of flavonoids and isoflavonoids, found as naturally occurring compounds, but it could be considered that their true importance is extended in two branches. The biological activity associated with them, including anti-inflammatory (Hsieh *et al.*, 2000; De Leon *et al.*, 2003), antipyretic (Mukherjee *et al.*, 2001), anti invasive (Park *et al.*, 1998), anticancer (Kumar *et al.*, 2003; Qian *et al.*, 2010), anti tuberculosis (Lahtchev *et al.*, 2008), and anti-fungal activities (Piotrowska *et al.*, 2011). And their recognized synthetic utility in the preparation of pharmacologically interesting heterocyclic systems like pyrazolines, which have been largely studied owing to their pharmacological activities, which includes anti-tumor, anti-inflammatory, anti-parasitary, anti-depressive, anti-convulsant, antimicrobial, and inflammatory arthritis (Johnson *et al.*, 2007; Ramana *et al.*, 2008; Bhat *et al.*, 2009; Ozdemir *et al.*, 2006).

Further, in recent times, it is reported that the incorporation of fluorine atom into heterocycles provides compounds with enhanced biological properties. The enhanced

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biological activity of fluorinated heterocycles is due to accumulation of fluorine on carbon and causing increased oxidative and thermal stability. Hence fluorinated drugs due to their inherent characteristics of being metabolically non-degradable and increased lipid solubility are utilized to enhance the rate of drug absorption and their in vivo transport (Lin *et al.*, 2003).

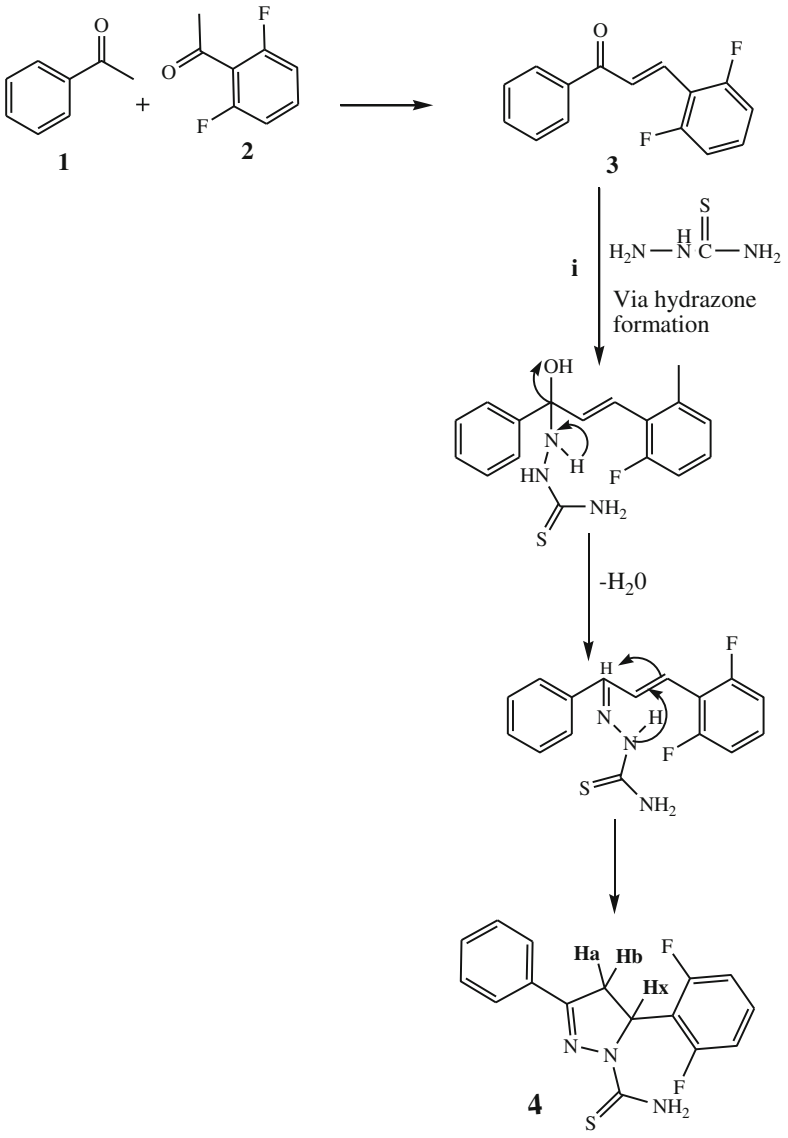
Recent reports suggest that pyrazoles are novel class of antitumor agents because of their focused anti proliferative and tumor-reducing activities (Lv *et al.*, 2010).

This renewed interest in this class of compounds and in continuation of our research to furnish biologically new active compounds (Shiradkar *et al.*, 2007; Mallikarjuna *et al.*, 2007, 2009; Suresh Kumar *et al.*, 2010a, b) has encouraged to synthesize a series of novel 4,5-dihydropyrazoles and evaluate their anticancer and antimicrobial activities.

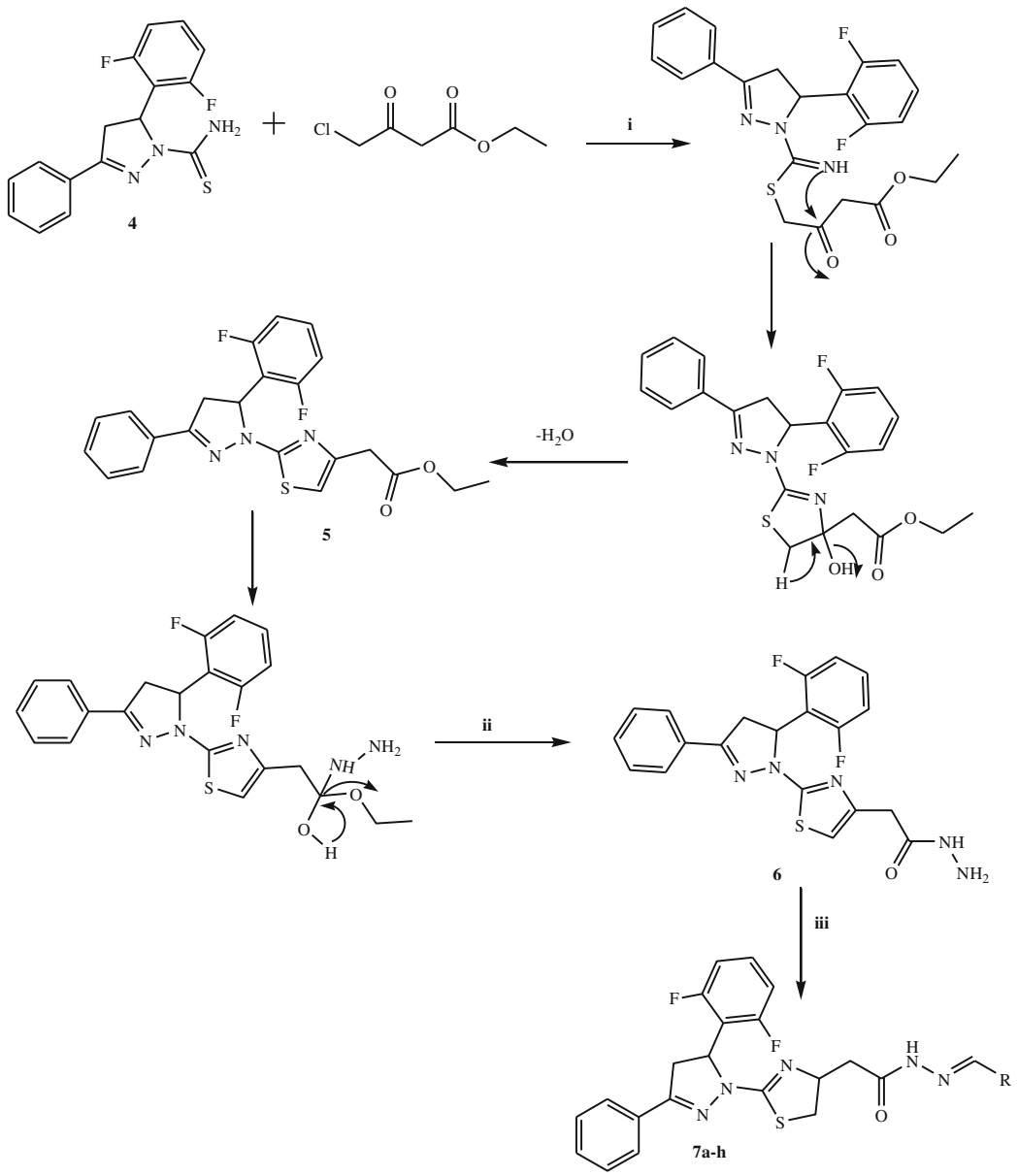
Chemistry

The reaction sequences employed for synthesis of target compounds are according to the literature (Holla *et al.*, 2000; Abdel-Wahab *et al.*, 2009; Budakoti *et al.*, 2009), and their reaction mechanisms are depicted in Schemes 1, 2, 3, and 4 and their physical properties are shown in Table 1.

Scheme 1 describes the synthesis of key intermediate compound **3** prepared according to the literature (Turan-Zitouni *et al.*, 2000; Chimenti *et al.*, 2010; Budakoti *et al.*, 2007; Szollosy *et al.*, 1991) by reacting acetophenone **1** and 2,6-difluoroacetophenone **2** by Claisen–Schmidt reaction. Compound **3** on reacting with thiosemicarbazide in basic conditions pursue reaction mechanism involving formation of intermediate (non-isolable) hydrazones and subsequent addition of NH on the carbon–carbon double

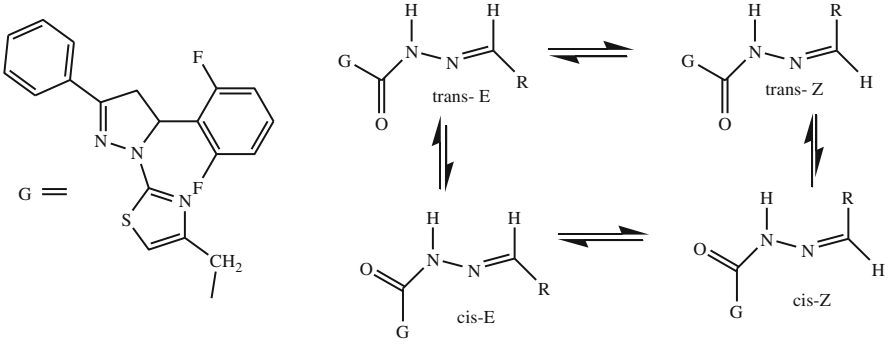


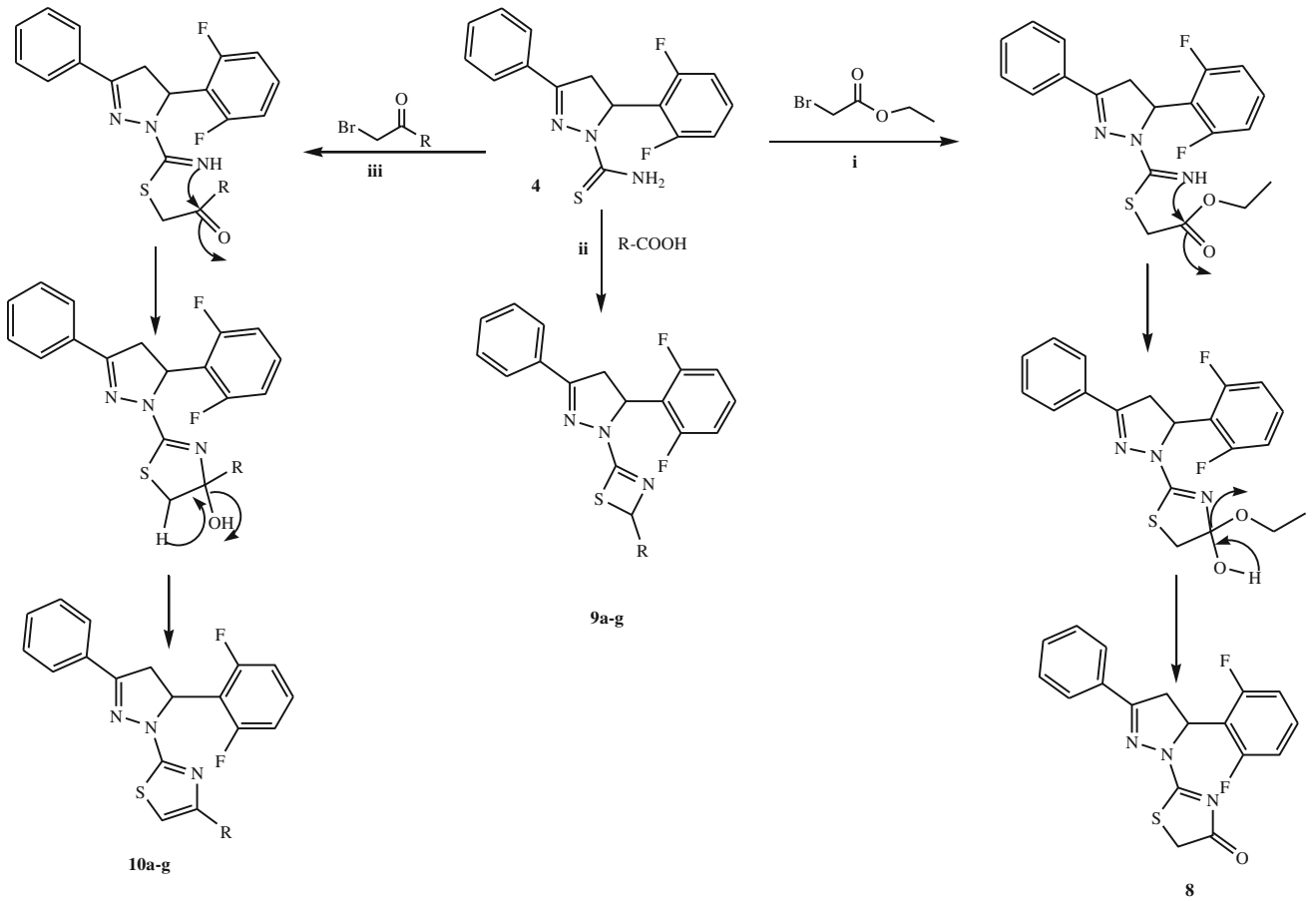
Scheme 1 Mechanism and synthesis of compound **4**. Conditions: (i) thiosemicarbazide, NaOH, ethanol, reflux 48 h



Scheme 2 Mechanism and synthesis of compound **6** and **7a-h**. Conditions: (i) ethyl chloro acetoacetate; (ii) hydrazine hydrate, ethanol, reflux 5 h; (iii) ethanol, substituted aldehyde, glacial acetic acid, reflux 3 h

Scheme 3 E/Z geometrical isomers and cis/transconfermers of **7a–h**





Scheme 4 Mechanism and synthesis of compound **8**, **9a-g** and **10a-g**. Conditions: (i) ethyl bromo acetate, ethanol reflux 1 h; (ii) substituted benzoic acid, $POCl_3$, reflux 5 h; (iii) substituted phenacyl bromides, ethanol, reflux 1 h

bond of the propenone moiety. The literature's apparent formation of 5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazole-1-carbothioamide **4**, hydrazine's attack preferentially on the carbonyl group of α,β-unsaturated ketones, rather than the double bond confirms the formation of compound **4** as racemic mixture by intermediate hydrazones mechanism.

Scheme 2 describes that compound **4** on reacting with ethyl chloroacetoacetate provides compound **5** via the non-isolable intermediates. 1-(2-(5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)-3-methoxypropan-2-one **5** on reacting with hydrazine hydrate provides compound **6**. The treatment of acetohydrazide derivative **6** with several aromatic aldehydes afford 2,6-(difluorobenzylidene)-2-(2-(5-substituted -3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl) acetohydrazides **7a**–**h**. The compounds **7a**–**h** comprising arylidenehydrazide structure may exist as E/Z geometrical isomers about –C=N double bond and as *cis*/*trans* amide conformers. (Scheme 3) According to the literature (Demirbas *et al.*, 2002, 2009; Salgin-Goksen *et al.*, 2007), the compounds containing imine bond are present in higher percentage in dimethyl-d_6 sulfoxide solution in the form of geometrical E isomer about –C=N double bond. The Z isomer can be stabilized in less polar solvents by an intramolecular hydrogen bond. In this study, the spectral data were obtained in dimethyl-d_6 sulfoxide solution, and no signal belonging to Z isomer was observed. On the other hand, the *cis*/*trans* conformers of E isomer were present in the dimethyl-d_6 sulfoxide solution of compounds **7a**–**h**.

Further, the aforementioned 1-thiocarbamoyl pyrazole derivative **4** were cyclized to pyrazolothiazol-4(5H)-ones **8** and pyrazolothiazole derivatives **10a**–**g** through their reaction with ethyl chloroacetate and phenacyl bromide derivatives, respectively, in hot ethanol for 1 h. While compound **4** on reacting with appropriate aromatic acids in presence of phosphorus oxychloride afforded thiazets **9a**–**g** as described in Scheme 4.

Biological activity

The standard strains were procured from the American Type Culture Collection (ATCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India. The

Table 1 Analytical and physico-chemical data of synthesized compounds (**3**, **4**, **5**, **6**, **7a–h**, **8**, **9a–g**, and **10a–g**)

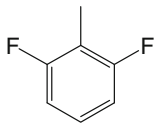
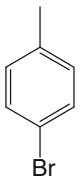
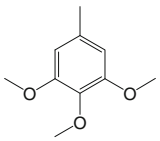
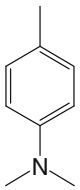
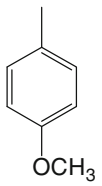
Compounds	R	Molecular formula	MW[a]	M.p. (°C)[b]/ crystallization solvent	Yield (%)	%Analysis of C, H, N found (calc.)[c]		
						C	H	N
3	–	$C_{15}H_{10}F_2O$	244.0	(135–138) ethanol	81.0	73.76 (73.72)	4.13 (4.11)	–
4	–	$C_{16}H_{13}F_2N_3S$	317.08	(144–147) ethanol	75.2	60.55 (60.53)	4.13 (4.11)	13.24 (13.22)
5	–	$C_{22}H_{19}F_2N_3O_2S$	427.12	(151–153) ethyl acetate/n-hexane	72.1	61.81 (61.82)	4.48 (4.44)	9.83 (9.87)
6	–	$C_{20}H_{17}F_2N_5OS$	413.0	(183–185) ethanol	70.3	58.10 (58.12)	4.14 (4.13)	16.94 (16.92)
7a		$C_{27}H_{19}F_4N_5OS$	537.1	(164–166) ethanol	85.8	60.33 (60.35)	3.56 (3.57)	13.03 (13.04)
7b		$C_{27}H_{20}BrF_2N_5OS$	580.05	(174–176) ethanol	83.2	55.87 (55.85)	3.47 (3.49)	12.07 (12.02)
7c		$C_{30}H_{27}F_2N_5O_4S$	591.18	(154–156) ethanol	86.4	60.90 (60.92)	4.60 (4.55)	11.84 (11.82)
7d		$C_{29}H_{26}F_2N_6OS$	544.19	(181–184) ethanol	79.0	63.95 (63.94)	4.81 (4.83)	15.43 (15.44)
7e		$C_{28}H_{23}F_2N_5O_2S$	531.15	(162–164) ethanol	77.5	63.26 (63.23)	4.36 (4.30)	13.17 (13.14)

Table 1 continued

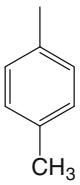
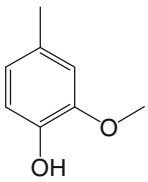
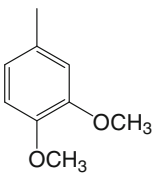
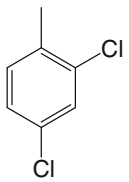
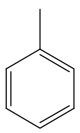
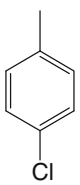
Compounds	R	Molecular formula	MW[a]	M.p. (°C)[b]/ crystallization solvent	Yield (%)	%Analysis of C, H, N found (calc.)[c]		
						C	H	N
7f		$C_{28}H_{23}F_2N_5OS$	515.16	(173–175) ethanol	82.1	65.23 (65.20)	4.50 (4.53)	13.58 (13.55)
7g		$C_{28}H_{23}F_2N_5O_3S$	547.15	(134–138) ethanol	80.4	61.42 (65.20)	4.23 (4.22)	12.79 (12.77)
7h		$C_{29}H_{25}F_2N_5O_3S$	561.16	(165–168) ethanol	82.1	62.02 (62.08)	4.49 (4.46)	12.47 (12.45)
8	–	$C_{18}H_{13}F_2N_3OS$	357.07	(225–228) ethanol	69.5	60.49 (60.48)	3.67 (3.69)	11.76 (11.74)
9a		$C_{23}H_{15}\ Cl_2F_2N_3S$	473.03	(238–241) ethyl acetate/*n*-hexane	84.1	58.24 (58.22)	3.19 (3.11)	8.86 (8.85)
9b		$C_{23}H_{17}F_2N_3S$	405.46	(245–247) ethyl acetate/*n*-hexane	81.9	68.13 (68.12)	4.23 (4.21)	10.36 (10.35)
9c		$C_{23}H_{16}ClF_2N_3S$	439.91	(212–216) ethyl acetate/*n*-hexane	74.3	62.80 (62.82)	3.67 (3.66)	9.55 (9.57)

Table 1 continued

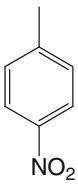
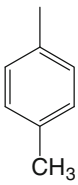
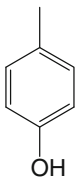
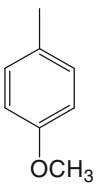
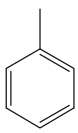
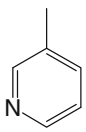
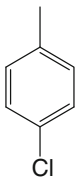
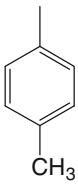
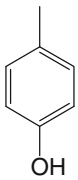
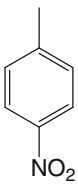
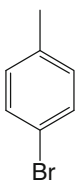
Compounds	R	Molecular formula	MW[a]	M.p. (°C)[b]/ crystallization solvent	Yield (%)	%Analysis of C, H, N found (calc.)[c]		
						C	H	N
9d		$C_{23}H_{16}F_2N_4O_2S$	450.1	(253–255) ethyl acetate/*n*-hexane	78.0	61.33 (61.32)	3.57 (3.58)	12.44 (12.47)
9e		$C_{24}H_{19}F_2N_3S$	419.13	(231–233) ethyl acetate/*n*-hexane	73.4	68.72 (68.71)	4.57 (4.49)	10.02 (10.07)
9f		$C_{23}H_{17}F_2N_3OS$	421.11	(247–249) ethyl acetate/*n*-hexane	83.2	65.54 (65.55)	4.07 (4.09)	9.97 (9.92)
9g		$C_{24}H_{19}F_2N_3OS$	435.12	(276–278) ethyl acetate/*n*-hexane	84.3	66.19 (66.15)	4.40 (4.41)	9.65 (9.66)
10a		$C_{24}H_{17}F_2N_3S$	417.11	(202–204) ethanol	80.3	69.05 (69.08)	4.10 (4.12)	10.07 (10.05)
10b		$C_{23}H_{16}F_2N_4S$	418.11	(215–217) ethanol	68.2	66.01 (66.03)	3.85 (3.82)	13.39 (13.37)

Table 1 continued

Compounds	R	Molecular formula	MW[a]	M.p. (°C)[b]/ crystallization solvent	Yield (%)	%Analysis of C, H, N found (calc.)[c]		
						C	H	N
10c		$C_{24}H_{16}ClF_2N_3S$	451.07	(224–226) ethanol	79.3	63.79 (63.78)	3.57 (3.56)	9.30 (9.32)
10d		$C_{25}H_{19}F_2N_3S$	431.13	(237–239) ethanol	75.0	69.59 (69.58)	4.44 (4.46)	9.74 (9.72)
10e		$C_{24}H_{17}F_2N_3OS$	433.11	(215–217) ethanol	79.3	66.50 (66.52)	3.95 (3.93)	9.69 (9.66)
10f		$C_{24}H_{16}F_2N_4O_2S$	462.1	(223–225) ethanol	73.0	62.33 (62.29)	3.49 (3.43)	12.11 (12.13)
10g		$C_{24}H_{16}BrF_2N_3S$	496.02	(275–276) ethanol	82.2	58.07 (58.08)	3.25 (3.23)	8.47 (8.43)

[a] Molecular weight of the compound

[b] Melting point of the compound

[c] Elemental analysis of C, H, and N were within ±0.4 % of theoretical value

antibacterial activity of the synthesized 4,5-dihydropyrazole derivatives (**4**, **5**, **6**, **7a–h**, **8**, **9a–g**, and **10a–g**) was performed by broth dilution method against the following standard bacterial strains *Staphylococcus aureus* (ATCC 11632), *Streptococcus faecalis* (ATCC 14506), *Bacillus subtilis* (ATCC 60511), *Klebsiella pneumoniae* (ATCC 10031), *Escherichia* and *Pseudomonas aeruginosa* (ATCC 10145) and antifungal activity against yeasts: *Saccharomyces cerevisiae* (ATCC 9763, Sc) and *Candida tropicalis* (ATCC 1369, CT), mould: *Aspergillus niger* (ATCC 6275).

Subsequently, evaluated for their in vitro anticancer activity against tumor cell lines panel consisted of Hela (human cervixcarcinoma cell line), A549 (human lung adenocarcinoma cell line), MCF-7 (human breast adenocarcinoma cell line), A2780 (human ovarian cancer cell line), and BGC-823 (human gastric cancer cell line) by using MTT assay Mosmann's method.

The MTT assay is based on the reduction of the soluble 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT 0.5 mg/mL, 100 μL), into a blue-purple formazan product, mainly by mitochondrial reductase activity inside living cells.

Results and discussion

Chemistry

The structures of all the synthesized compounds were inferred from elemental analysis, mass spectrometry, IR, ^{1}H, and ^{13}C NMR substantiating in full agreement with those reported in the literature (Turan-Zitouni *et al.*, 2000; Chimenti *et al.*, 2010; Budakoti *et al.*, 2007; Szollosy *et al.*, 1991). The IR spectra of the compound **4** reveal absorption bands at 1,592 cm^{-1} corresponding to C=N stretching bands because of ring closure. In ^{1}H NMR spectra, the two methylene protons (Ha and Hb) at position C_4 are geminal protons and appears in the region δ 3.24–3.28 and 3.80–3.85 ppm as doublet of doublets, respectively, with J_{AB} 17.01 Hz. The CH proton (H_x) at C_5 position appears as doublet of doublets in the region of δ 5.95–5.98 ppm with different J values ($J_{Ax} = 5.2$ Hz, $J_{Bx} = 11.02$), due to vicinal coupling of two non-equivalent geminal protons of C_4 carbon. In the ^{13}C NMR spectra, the C_4 and C_5 carbons of the pyrazoline ring in compound **4** resonated at 37.12 and 61.39 ppm, respectively, indicating not only the formation of the pyrazoline, but also the exact position of the C=N double bond.

The protons belonging to the aromatic ring and the other aliphatic groups were observed with the expected chemical shift and integral values. In MS spectra, the fragment peaks which correspond to loss of –SH, –NH_2, –$CSNH_2$ from the molecular ion are consistent with the postulated structure. Characteristic M+2 isotope peaks are observed in the mass spectra of the compounds having a sulfur or halogen. Further, the molecular ion peak *m/z* 317.08 in mass spectrum of compound **4** was found to be in conformity with its molecular formula of the assigned structure.

The 2,6-difluorophenyl)-3-phenyl-4, 5-dihydropyrazol-1-yl) thiazol-4-yl-substituted acetohydrazide derivatives **7a–h** was established by lack of signals corresponding to NH_2 of structure **6** in ^{1}H NMR spectra. Appearance of peaks corresponding to N=CH in ^{13}C NMR spectra and signals due to aromatic hydrogen's in range of δ 6.32 to 7.78 in ^{1}H NMR spectra confirmed the formation of these analogues. Further, ^{1}H NMR, ^{13}C NMR, mass spectra, and elemental analysis supported the structures of synthesized acetohydrazide derivatives **7a–h**.

The structure of the new thiazol-4(5H)-one **8** were confirmed using IR spectra which showed strong absorption bands at 1,696 cm^{-1} due to carbonyl group. In addition, ^{1}H NMR revealed singlet signals at around 3.98 ppm integrating two protons of the thiazolone ring and the disappearance of the exchangeable signals of the amino group protons. ^{13}C NMR confirmed the proposed structure due to the appearance of signal at 187.6 ppm due to carbonyl carbon as well as the appearance of signal around 39.04 ppm assignable to C_5 of the thiazolone ring.

The structure of compound **9a** was confirmed by lack of resonances corresponding to C=S in ^{13}C NMR and appearance of peaks at 6.32 due to thiazide formation in ^{1}H NMR spectra. Further, mass spectrum divulge molecular ion peak at *m/z* 473.03 which is in agreement with the molecular formula $C_{23}H_{15}Cl_2F_2N_3S$ of compound **9a**.

The structures of the new thiazole derivatives **10a–g** were characterized using ^{1}H NMR spectra which revealed the presence of singlet peak at δ 6.51–6.94 ppm assignable to C_5–H thiazole. And appearance of characteristic peaks in ^{13}C NMR spectra corresponding to thiazole ring at around 161.62–165.71 (C_2 of thiazole), 154.25–159.61 (C_4 of thiazole) indicates the formation of thiazole ring and molecular ion peak *m/z* at 417.11 in mass spectrum confirm the formation of compound **10a**.

Pharmacological activity and structure activity relationship

The structure activity relationship (SAR) studies in earlier communication illustrated that difluoro-substituted heterocycles exhibit excellent antimicrobial inhibition; this increased activity was attributed to presence of fluorine atoms (highly electro negative) in the molecule which increases the liphophilicity and affects the partitioning of a molecule into the membranes and facilitates hydrophobic interactions of the molecule with specific binding sites on either receptor or enzymes (Suresh Kumar *et al.*, 2010a, b).

The results of antimicrobial testing of synthesized compounds (**4**, **5**, **6**, **7a–h**, **8**, **9a–g**, and **10a–g**) against selected Gram-positive, Gram-negative bacteria, yeasts, moulds, and anticancer activity against human tumor cells Hela (Human cervix carcinoma cell line), A549 (Human lung adenocarcinoma cell line), MCF-7 (Human breast adenocarcinoma cell line), A2780 (Human ovarian cancer cell line) and BGC-823 (Human gastric cancer cell line) are illustrated in Tables 2 and 3, respectively.

The antimicrobial activity of the series **7a–h** revealed that compound **7a** comprising 2,6-difluorosubstitution exhibited excellent anticancer activity and antimicrobial inhibition against tested microbial species. Compound **7f** consisting of electron-donating *p*-CH_3 depicts excellent antifungal inhibition, but loss of activity against tested bacterial species. This concludes that the effect of the substituent at the *para*-position is likely to be related to the size of the substituent with small and electron-withdrawing substituent (Cl or F) play an important role for the activity.

The antimicrobial activity of 4-substituted-1,3-thiazet-2-yl-5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole derivatives **9a–g** consisting diverse substitutions

Table 2 Antimicrobial activity expressed as MIC (μg/mL)

Compounds	Gram-positive organisms[a]			Gram-negative organisms[b]			Fungi[c]		
	Sa	Sf	Bs	Kp	Ec	Pa	Sc	Ct	An
3	31.25	125	125	125	125	8	62.5	62.5	62.5
4	31.25	31.25	8	62.5	62.5	31.25	125	31.25	62.5
5	16	31.25	31.25	8	8	8	62.5	31.25	62.5
6	31.25	62.5	62.5	31.25	16	31.25	31.25	8	31.25
7a	16	16	62.5	8	4	8	16	4	16
7b	8	31.25	8	4	4	16	8	31.25	125
7c	31.25	125	31.25	16	16	31.25	31.25	31.25	16
7d	31.25	31.25	62.5	31.25	62.5	62.5	31.25	62.5	16
7e	250	125	16	125	16	125	250	125	125
7f	125	16	16	125	125	16	8	8	16
7g	8	16	16	16	62.5	62.5	62.5	31.25	125
7h	125	125	16	16	125	31.25	8	16	16
8	16	16	31.25	16	62.5	16	31.25	31.25	31.25
9a	125	125	31.25	31.25	16	125	16	62.5	8
9b	16	8	31.25	16	16	62.5	62.5	31.25	125
9c	31.25	125	31.25	16	8	62.5	4	8	8
9d	31.25	16	62.5	31.25	31.25	8	8	125	125
9e	16	8	16	4	8	4	62.5	125	62.5
9f	16	31.25	31.25	16	4	4	16	16	31.25
9g	16	16	4	4	16	4	16	8	8
10a	31.25	8	8	62.5	31.25	62.5	62.5	125	125
10b	31.25	31.25	31.25	31.25	16	16	31.25	16	16
10c	62.5	8	31.25	125	16	31.25	125	62.5	62.5
10d	4	8	4	4	8	8	62.5	125	62.5
10e	16	125	31.25	16	62.5	62.5	31.25	125	16
10f	16	16	16	16	125	31.25	125	125	16
10g	16	8	8	16	31.25	31.25	62.5	62.5	62.5
Ciprofloxacin	≤5	≤5	≤1	≤1	≤1	5	–	–	–
Norfloxacin	5	5	≤1	≤1	≤1	5	–	–	–
Flucanozole	–	–	–	–	–	–	≤1	≤1	≤1

[a] The screening organisms. Gram-positive bacteria: *Staphylococcus aureus* (ATCC 11632, Sa), *Streptococus faecalis* (ATCC 14506, Sf), and *Bacillus subtilis* (ATCC 60511, Bs)

[b] The screening organisms. Gram-negative bacteria: *Klebsiella penumoniae* (ATCC 10031, Kp), *Escherichia coli* (ATCC 10536, Ec), and *Pseudomonas aeruginosa* (ATCC 10145, Pa)

[c] The screening organisms. Yeasts: *Saccharomyces cerevisiae* (ATCC 9763, Sc) and *Candida tropicalis* (ATCC 1369, Ct), mould: *Aspergillus niger* (ATCC 6275, An)

Table 3 Cytotoxicity of synthesized compounds (**3**, **4**, **5**, **6**, **7a–h**, **8**, **9a–g**, and **10a–g**) against human tumor cells (IC50 ± SD, μM)

Compounds	Human tumor cells				
	Hela	A549	MCF-7	A2780	BGC-823
4	6.25 ± 1.24	2.55 ± 0.15	2.15 ± 2.10	3.62 ± 1.29	1.84 ± 0.72
5	6.31 ± 3.29	4.18 ± 0.24	2.11 ± 0.54	3.91 ± 1.54	3.85 ± 0.38
6	6.88 ± 1.44	1.84 ± 0.32	2.02 ± 0.41	3.88 ± 1.69	1.95 ± 0.71
7a	**4.23 ± 0.39**	**0.96 ± 0.28**	**1.20 ± 0.92**	2.54 ± 0.48	1.32 ± 0.53
7b	5.98 ± 0.50	4.14 ± 0.37	1.85 ± 0.47	3.89 ± 0.45	1.56 ± 0.65
7c	7.04 ± 1.13	2.30 ± 0.43	192 ± 0.50	4.30 ± 0.42	1.09 ± 0.56
7d	7.87 ± 0.08	2.87 ± 0.52	1.85 ± 0.34	3.44 ± 0.70	1.76 ± 0.71
7e	6.30 ± 0.32	1.84 ± 0.48	1.74 ± 0.43	3.57 ± 0.46	1.54 ± 0.39
7f	5.91 ± 0.81	1.50 ± 0.72	1.65 ± 0.53	2.62 ± 0.44	1.45 ± 0.28
7g	7.23 ± 0.47	4.48 ± 0.84	2.12 ± 0.49	2.78 ± 0.56	1.22 ± 0.37
7h	6.09 ± 0.33	1.89 ± 0.42	2.60 ± 0.48	3.93 ± 0.57	1.28 ± 0.30
8	6.45 ± 1.84	4.58 ± 0.11	1.94 ± 0.21	5.32 ± 1.25	2.35 ± 0.16
9a	6.13 ± 0.35	3.25 ± 0.87	1.89 ± 0.35	4.51 ± 2.32	3.31 ± 0.54
9b	5.71 ± 0.24	2.11 ± 0.64	1.72 ± 1.98	4.33 ± 1.59	1.97 ± 0.91
9c	**4.94 ± 0.91**	1.56 ± 0.15	1.69 ± 1.14	2.72 ± 0.41	1.41 ± 0.29
9d	6.62 ± 0.56	3.26 ± 0.95	3.22 ± 2.05	4.35 ± 0.36	6.11 ± 0.73
9e	6.59 ± 0.75	4.21 ± 0.51	3.23 ± 0.85	5.14 ± 0.87	3.22 ± 0.24
9f	6.23 ± 0.41	3.44 ± 0.23	4.32 ± 0.11	3.14 ± 0.23	1.04 ± 0.34
9g	6.49 ± 0.19	2.53 ± 0.56	2.04 ± 0.43	3.26 ± 0.87	1.23 ± 0.89
10a	5.91 ± 0.47	3.01 ± 0.50	2.23 ± 0.50	3.09 ± 1.34	2.01 ± 0.04
10b	5.98 ± 0.24	1.43 ± 0.35	2.45 ± 0.52	3.18 ± 1.52	1.11 ± 1.11
10c	6.24 ± 0.48	2.11 ± 0.41	2.76 ± 0.47	3.56 ± 0.34	1.43 ± 1.22
10d	**4.86 ± 0.49**	**1.21 ± 0.92**	2.50 ± 0.42	3.03 ± 1.54	1.20 ± 0.56
10e	7.09 ± 0.53	1.57 ± 0.08	3.04 ± 0.58	2.45 ± 1.02	2.11 ± 0.43
10f	5.76 ± 0.60	1.93 ± 0.59	2.09 ± 0.44	2.26 ± 0.64	1.05 ± 0.30
10g	6.12 ± 0.77	1.93 ± 0.52	2.14 ± 0.58	2.45 ± 0.70	1.34 ± 0.59
Cisplatin (control)	5.71 ± 0.57	1.33 ± 0.55	1.62 ± 0.44	2.32 ± 0.39	0.92 ± 0.19

[a] Mean value ± SD (standard deviation from three experiments)

[b] Boldface: IC50 ≤ the control

with varying degrees of electronic and spatial arrangements divulged imperative structural activity relationship data. Among the electron-donating derivatives, compound **9g** comprising (R = OMe) strong electron-donating group at the C-4 position of the phenyl ring showed improved antimicrobial activity against tested bacterial and fungal species, but exhibited decreased antitumor activity.

Compounds **9e** and **9f** possessing *p*-CH_3 and *p*-OH substitution on phenyl ring exhibited moderate antimicrobial activity against Gram-positive species and excellent activity against tested Gram-negative species *K. penumoniae* and *E. coli* than *P. aeruginosa*.

The compound **9c** having 4-chloro substitution showed excellent anticancer (IC50 4.94 μM against Hela (human cervix carcinoma cell line), which implied that the lipophilic and electron-withdrawing halobenzyl groups were beneficial for the cytotoxic activity against the Hela cell lines.

Antimicrobial activity of thiazole derivatives **10a–g** revealed that this series of compounds was more effective against the Gram-positive bacteria with MIC 4 to 31.25 μg/mL. Particularly, 4-methyl substituted derivative **10d** exhibited excellent inhibition at MIC 4–8 μg/mL against tested Gram-positive bacteria.

In contradiction compound **10b** comprising pyridine substitution showed moderate to good inhibition against tested Gram-negative organisms and excellent anticancer activity. Compounds **10c** and **10e** which possess inductively electron withdrawing but mesomerically electron-donating substituent's on phenyl group were found to be less active compounds against the tested against human tumor cells.

Conclusion

In conclusion, this work demonstrates the synthesis of novel series of 4,5-dihydropyrazole derivatives (**4**, **5**, **6**, **7a–h**, **8**, **9a–g**, and **10a–g**) and in vitro evaluation of their antimicrobial (bacterial and fungal) and anticancer activity against Hela (human cervix carcinoma cell line), A549 (human lung adenocarcinoma cell line), MCF-7 (human breast adenocarcinoma cell line), A2780 (human ovarian cancer cell line), and BGC-823 (human gastric cancer cell line) by using MTT assay.

Antimicrobial study revealed that compounds **7a**, **9c**, **9g**, and **10d** demonstrated significant activity against tested Gram-positive and Gram-negative bacteria and fungal species. The in vitro anticancer screening of the synthesized series illustrate that all compounds were active, in particular, compounds **7a**, **9c**, and **10d** exhibited excellent anticancer activity when compared with reference drug cisplatin. The promising in vitro antimicrobial and anticancer activity of flouro-substituted dihydropyrazole derivatives make them certainly promising molecules for further lead optimization in the development of novel antimicrobial and anticancer agents.

Experimental

Chemical protocols

Melting points were determined in open capillary tubes in a Thomas Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded on Shimadzu FT-IR 157, ^{1}H NMR, and ^{13}C NMR spectra were recorded (in CDCl3/DMSO-d_6) on a Bruker spectrometer at 300/400 MHz using TMS as an internal standard. Mass spectra (EI) on (AMD-604) mass spectrometer operating at 70 eV. Elemental analysis was performed on Thermo Finnigan Flash (EA 1112 CHNS Analyzer).

*Synthesis of 5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazole-1-carbothioamide (**4**)*

To a suspension of chalcone **3** (0.01 mol) and sodium hydroxide (0.025 mol) in ethanol (50 mL), thiosemicarbazide (0.01 mol) was added and the mixture was refluxed for 48 h. The product obtained was poured into crushed ice and the solid mass which separated out was filtered, dried, and crystallized with appropriate solvent.

IR (KBr) ν max, cm^{-1}: (C = S) 1334, (C=N) 1592, (NH_2) 3280 ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.24–3.28 (dd, 1H, $J_{AB} = 17.01$, $J_{Ax} = 5.2$, C_4–H_A of pyrazole), 3.80–3.85 (dd, 1H, $J_{Bx} = 11.02$, C_4–H_B of pyrazole), 5.95–5.98 (dd, 1H, C_5–H_x of pyrazole), 6.74–7.62 (m, 10H, ArH + NH_2) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 37.12, 61.39 (C_4, C_5 of pyrazole), 114.35–156.22 (phenyl-C), 156.04 (C=N), 186.55 thiocarbamoyl carbon (C=S) ppm. *m/e*: 317.08 (100.0 %), 318.08 (19.2 %), 319.08 (4.9 %).

*Synthesis of ethyl 2-(2-(5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)acetate (**5**)*

To a suspension of compound **4** (0.01 mol) in ethanol (20 mL), ethyl chloro acetoacetate (0.01 mol) were added and heated at reflux for 1 h. After cooling, the product was collected by filtration and crystallized from an appropriate solvent.

IR (KBr) ν max, cm^{-1}: (C–O) 1330 ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.13 (t, 3H, OCH_2–CH_3), 3.23–3.26 (dd, 1H, $J_{AB} = 17.02$, $J_{Ax} = 5.1$, C_4–H_A of pyrazole), 3.63 (s, 2H, OCH_2–CH_3), 3.82–3.86 (dd, 1H, $J_{Bx} = 11.04$, C_4–H_B of pyrazole), 4.13 (m, 2H, CH_2), 5.92–5.95 (dd, 1H, C_5–H_x of pyrazole), 6.13 (s, 1H, CH, thiazole), 6.95–7.80 (m, 8H, ArH) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 39.1 (CH_2), 14.13 (OCH_2–CH_3), 58.58 (OCH_2–CH_3), 172.21 (C=O), 104.3, 154.6, 164.3 (C_2, C_3 C_5 thiazole), 113.35–156.22 (phenyl-C), 161.22, 43.33, 62.43 (C_3, C_4, C_5 of pyrazole) ppm. *m/e*: 431.04 [(M+4)$^+$ 6.38 %], 429.2 [(M+2)$^+$ 20.3 %], 427.12 (M^+ 100.0 %), 428.12 (24.9 %).

*Synthesis of 2-(2-(5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)acetohydrazide (**6**)*

The mixture of compound **5** (0.015 mol) and hydrazine hydrate (1.6 mL) in absolute ethanol (20 mL) was refluxed for 5 h. The mixture was cooled and the crystalline mass obtained was recrystallised. IR (KBr) ν max, cm^{-1}: 1610 (C=0). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.26–3.28 (dd, 1H, J_{AB} 17.01, $J_{Ax} = 5.05$, C_4–H_A of pyrazole), 3.62 (s, 2H, CH_2–C=O), 3.85–3.87 (dd, 1H, $J_{Bx} = 11.04$, C_4–H_B of pyrazole), 4.51 (s, 2H, NH–NH_2 disappeared on D_2O exchange), 6.24 (s, 1H, CH, thiazole), 5.95–5.97 (dd, 1H, C_5–H_x of pyrazole), 6.95–7.80 (m, 8H, ArH), 9.82 (s, 1H, NH-NH_2 disappeared on D_2O exchange) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 39.8 (CH_2–C=O), 121.11–152.45 (phenyl-C), 103.5, 152.1, 167.6 (C_2, C_3, C_5 thiazole), 160.34, 38.23, 61.22 (C_3, C_4, C_5 of pyrazole), 189.4 (C=O) ppm. *m/e*: 413.23 (100.0 %), 413.12 (20.7 %), 415.13 (5.4 %), 414.11 (2.6 %), 415.12 (2.5 %).

*General synthesis of synthesis of 2-(2-(5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)-N′-substituted acetohydrazide derivatives (**7a–h**)*

Equimolar quantities of compound **6** and substituted aromatic aldehydes were refluxed in alcohol for 3 h in the presence of few drops of glacial acetic acid. The solvent

was evaporated and the product was poured on cold water, filtered, and dried. The crude solid was recrystallised in appropriate solvent systems to give the products.

*Synthesis of (2,6-difluorobenzylidene)-2-(2-(5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl) acetohydrazide (**7a**)* IR (KBr) ν max, cm^{-1}: 3210 (NH), 1628 (amide C=O), 1670 (amide C=N). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 6.62–7.72 (m, 12H, Ar–H + C_5–H of thiazole), 3.28–3.32 (dd, 1H, $J_{AB} = 17.04$, $J_{Ax} = 5.08$, C_4–H_A of pyrazole), 3.98–4.02 (dd, 1H, $J_{Bx} = 11.04$, C_4–H_B of pyrazole), 4.58 and 4.18 (s, 2H, CH_2 *trans*/*cis* conformers), 5.84–5.87 (dd, 1H, C_5–H_x of pyrazole), 8.14 and 7.98 (s, 1H, N=*CH trans*/*cis* conformers), 11.72 and 11.68 (s, 1H, NH, *trans*/*cis* conformers) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 41.24 and 41.89 (CH_2, *trans*/*cis* conformers) 44.2, 63.1 (C_4, C_5, pyrazole),105.14,156.76,165.71 (C_2, C_3, C_5 of thiazole), 118.1–134.2 (phenyl-C), 151.92 (C=N pyrazole), 149.1 and 149.82 (N=CH *trans*/*cis* conformers), 164.11(CH_2–*C*=*O*) ppm. *m*/*e*: 539.62 [$(M+2)^+$], 537.12 (100.0 %), 538.13 (29.5 %), 539.13 (5.2 %).

*Synthesis of N′-(4-bromobenzylidene)-2-(2-(5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl) acetohydrazide (**7b**)* IR (KBr) ν max, cm^{-1}: 3196 (NH), 1642 (amide C=O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.32–3.36 (dd, 1H, $J_{AB} = 17.04$, $J_{Ax} = 5.04$, C_4–H_A of pyrazole), 3.92–3.96 (dd, 1H, $J_{Bx} = 11.04$, C_4–H_B of pyrazole), 4.56 and 4.14 (s, 2H, CH_2 *trans*/*cis* conformers), 5.86–5.89 (dd, 1H, C_5–H_x of pyrazole), 6.54–7.78 (m, 13H, Ar–H + C_5–H of thiazole), 8.12 and 7.94 (s, 1H, N=*CH trans*/*cis* conformers), 11.71 and 11.67 (s, 1H, NH, *trans*/*cis* conformers), ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 41.22 and 41.84 (CH_2, *trans*/*cis* conformers) 44.52, 66.55 (C_4, C_5, pyrazole), 104.36, 154.81, 164.43 (C_2, C_3, C_5 of thiazole), 116.1–151.74 (phenyl-C), 149.24 and 149.89 (N=CH *trans*/*cis* conformers), 152.96 (C=N pyrazole), 165.22(CH_2–*C*=*O*) ppm. *m*/*e*: 581.05 (100.0 %), 579.05 (97.7 %), 580.05 (2.6 %), 584.05 (1.3 %).

*Synthesis of N′-(3,4,5-trimethoxybenzylidene)-2-(2-(5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl) acetohydrazide (**7c**)* IR (KBr) ν max, cm^{-1}: 3198 (NH), 1647 (amide C=O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 6.52–7.54 (m, 11H, Ar–H + C_5–H of thiazole), 3.34–3.38 (dd, 1H, $J_{AB} = 17.04$, $J_{Ax} = 5.04$, C_4–H_A of pyrazole), 3.90–3.92 (dd, 1H, $J_{Bx} = 11.04$, C_4–H_B of pyrazole), 4.52 and 4.11 (s, 2H, CH_2 *trans*/*cis* conformers), 5.84–5.92 (dd, 1H, C_5–H_x of pyrazole), 8.12 and 7.94 (s, 1H, N=CH *trans*/*cis* conformers), 11.58 and 11.62 (s, 1H, NH, *trans*/*cis* conformers) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 111.1–157.51 (phenyl-C), 104.26, 155.87, 162.43 (C_2, C_3, C_5 of thiazole), 152.54 (C=N pyrazole), 43.65, 64.58 (C_4, C_5, pyrazole), 56.2 (3-OCH_3), 149.05 and 149.86 (N=CH *trans*/*cis* conformers), 41.32 and 41.95 (CH_2, *trans*/*cis* conformers) 165.22(CH_2–*C*=*O*) ppm.

*Synthesis of N′-(4-(dimethylamino)benzylidene)-2-(2-(5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl) thiazol-4-yl)acetohydrazide (**7d**)* IR (KBr) ν max, cm^{-1}: 3193 (NH), 1642 (amide C=O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.85 (s, 6H, N-$2CH_3$), 3.32–3.36 (dd, 1H, $J_{AB} = 17.04$, $J_{Ax} = 5.07$, C_4–H_A of pyrazole), 3.90–3.96 (dd, 1H, $J_{Bx} = 11.08$, C_4–H_B of pyrazole), 4.58 and 4.18 (s, 2H, CH_2 *trans*/*cis* conformers), 5.82–5.86 (dd, 1H, C_5–H_x of pyrazole), 6.41–7.62 (m, 13H, Ar–H + C_5–H of thiazole), 8.14 and 7.98 (s, 1H, N=*CH trans*/*cis* conformers), 11.72 and 11.68 (s, 1H, NH, *trans*/*cis* conformers) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 41.26 and 41.88 (CH_2, *trans*/*cis* conformers), 44.85, 64.78 (C_4, C_5, pyrazole), 105.11, 154.27, 163.25 (C_2, C_3, C_5 of thiazole), 121.1–156.25 (phenyl-C), 40.32 (N-$2CH_3$),149.01 and 149.78 (N=CH *trans*/*cis* conformers), 152.54 (C=N pyrazole), 168.62(CH_2–*C*=*O*) ppm.

*Synthesis of N′-(4-methoxybenzylidene)-2-(2-(5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl) acetohydrazide (**7e**)* IR (KBr) ν max, cm^{-1}: 3197 (NH), 1640 (amide C=O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.34–3.38 (dd, 1H, $J_{AB} = 17.04$, $J_{Ax} = 5.07$, C_4–H_A of pyrazole), 3.73 (s, 3H, OCH_3), 3.92–3.96 (dd, 1H, $J_{Bx} = 11.08$, C_4–H_B of pyrazole), 4.58 and 4.27 (s, 2H, CH_2 *trans*/*cis* conformers), 5.84–5.88 (dd, 1H, C_5–H_x of pyrazole), 6.32–7.78 (m, 13H, Ar–H + C_5–H of thiazole), 8.16 and 7.94 (s, 1H, N=*CH trans*/*cis* conformers), 11.76 and 11.55 (s, 1H, NH, *trans*/*cis* conformers) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 41.24 and 41.89 (CH_2, *trans*/*cis* conformers), 43.85, 64.78 (C_4, C_5, pyrazole), 55.92 (–OCH_3 of p-methoxy phenyl), 104.71, 156.77, 164.18 (C_2, C_3, C_5 of thiazole), 125.1–158.45 (phenyl-C), 155.74 (C=N pyrazole), 149.15 and 149.91 (N=CH *trans*/*cis* conformers), 168.62(CH_2–*C*=*O*) ppm.

*Synthesis of N′-(4-tolylbenzylidene)-2-(2-(5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl) acetohydrazide (**7f**)* IR (KBr) ν max, cm^{-1}: 3198 (NH), 1641 (amide C=O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.33 (s, 3H, CH_3), 3.34–3.38 (dd, 1H, $J_{AB} = 17.04$, $J_{Ax} = 5.07$, C_4–H_A of pyrazole), 3.92–3.96 (dd, 1H, $J_{Bx} = 11.08$, C_4–H_B of pyrazole), 4.58 and 4.18 (s, 2H, CH_2 *trans*/*cis* conformers),5.84–5.88 (dd, 1H, C_5–H_x of pyrazole), 6.32–7.78 (m, 13H, Ar–H + C_5–H of thiazole), 8.14 and 7.98 (s, 1H, N=*CH trans*/*cis* conformers), 11.76

and 11.69 (s, 1H, NH, *trans*/*cis* conformers) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 24.32 (–CH_3 of p-methyl phenyl), 41.32 and 41.94 (CH_2, *trans*/*cis* conformers), 43.85, 64.78 (C_4, C_5, pyrazole), 104.71, 156.77, 164.18 (C_2, C_3, C_5 of thiazole), 125.1–158.45 (Phenyl-C), 149.24 and 149.94 (N=CH *trans*/*cis* conformers), 155.74 (C=N pyrazole), 168.62(CH_2–*C*=*O*) ppm.

*Synthesis of N′-(4-hydroxy-3-methoxybenzylidene)-2-(2-(5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl) thiazol-4-yl)acetohydrazide (**7g**)* IR (KBr) ν max, cm^{-1}: 3199 (NH), 1642 (amide C=O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.34–3.38 (dd, 1H, J_{AB} 17.04, J_{Ax} = 5.07, C_4–H_A of pyrazole), 3.79 (s, 3H, -OCH_3), 3.92–3.96 (dd, 1H, J_{Bx} = 11.08, C_4–H_B of pyrazole), 4.67 and 4.26 (s, 2H, CH_2 *trans*/*cis* conformers), 5.12 (s, 1H, OH), 5.84–5.88 (dd, 1H, C_5–H_x of pyrazole), 6.32–7.78 (m, 12H, Ar–H + C_5–H of thiazole), 8.23 and 8.04 (s, 1H, N=*CH trans*/*cis* conformers), 11.68 and 11.84 (s, 1H, NH, *trans*/*cis* conformers) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 41.22 and 41.89 (CH_2, *trans*/*cis* conformers), 43.85, 64.78 (C_4, C_5, pyrazole), 56.22 (–OCH_3),104.71, 156.77, 164.18 (C_2, C_3, C_5 of thiazole), 125.1–158.45 (Phenyl-C), 155.74 (C=N pyrazole), 149.31 and 149.86 (N=CH *trans*/*cis* conformers), 168.62(CH_2–*C*=*O*) ppm. *m*/*e*: 549.25 [(M+2)$^+$], 547.15 (100.0 %), 548.15 (33.0 %), 549.16 (4.5 %).

*Synthesis of N′-(3,4-dimethoxybenzylidene)-2-(2-(5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)acetohydrazide (**7h**)* IR (KBr) ν max, cm^{-1}: 3192 (NH), 1640 (amide C=O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 6.32–7.78 (m, 12H, Ar–H + C_5–H of thiazole), 3.34–3.38 (dd, 1H, J_{AB} = 17.04, J_{Ax} = 5.07, C_4–H_A of pyrazole), 3.73 (s, 6H, 2–OCH_3), 3.92–3.96 (dd, 1H, J_{Bx} = 11.08, C_4–H_B of pyrazole), 4.58 and 4.18 (s, 2H, CH_2 *trans*/*cis* conformers), 5.84–5.88 (dd, 1H, C_5–H_x of pyrazole), 8.16 and 8.02 (s, 1H, N=*CH trans*/*cis* conformers), 11.76 and 11.69 (s, 1H, NH, *trans*/*cis* conformers) ppm. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 6.32–7.78 (m, 12H, Ar–H + C_5–H of thiazole), 3.34–3.38 (dd, 1H, J_{AB} = 17.04, J_{Ax} = 5.07, C_4–H_A of pyrazole), 3.73 (s, 6H, 2-OCH_3), 3.92–3.96 (dd, 1H, J_{Bx} = 11.08, C_4–H_B of pyrazole), 4.58 and 4.18 (s, 2H, CH_2 *trans*/*cis* conformers), 5.84–5.88 (dd, 1H, C_5–H_x of pyrazole), 8.16 and 8.02 (s, 1H, N=CH *trans*/*cis* conformers), 11.76 and 11.69 (s, 1H, NH, *trans*/*cis* conformers) ppm.

*Synthesis of 2-(5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4(5H)-one (**8**)*

To a suspension of compound **4** (0.01 mol) in ethanol (20 mL), ethyl bromo acetate (0.01 mol) was added and refluxed for 1 h. After cooling, the separated product was filtered and washed. The product was crystallized from appropriate solvent.

IR (KBr) ν max, cm^{-1}: (C=O) 1696. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.25–3.27 (dd, 1H, J_{AB} = 17.05, J_{Ax} = 5.11, C_4–H_A of pyrazole), 3.82–3.84 (dd, 1H, J_{Bx} = 11.04, C_4–H_B of pyrazole), 3.98 (s, 2H, C_5–H of thiazolone), 5.92–5.94 (dd, 1H, C_5–H_x of pyrazole), 6.82–7.71 (m, 8H, ArH) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 39.04 (C_5 of thiazolone), 117.11–156.21 (phenyl-C), 187.61 (C=O), 157.65 (C–O), 161.22, 37.21, 63.12 (C_3, C_4, C_5 of pyrazole), 177.34 (C=N, thiazolone) ppm. *m*/*e*: 359.91 [(M+2)$^+$], 357.07 (100.0 %), 359.07 (4.5 %), 359.08 (2.4 %).

*General synthesis of 1-(4-substituted-1,3-thiazet-2-yl)-5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole derivatives (**9a**–**g**)*

An equimolar mixture (0.1 mol) of compound **4** and appropriate aromatic acids in phosphorus oxychloride (10 mL) was refluxed for 5 h. Excess of phosphorus oxychloride was removed under reduced pressure. The reaction mixture was cooled to room temperature and then gradually poured on to crushed ice with stirring. The mixture was allowed to stand overnight and the solid separated out was filtered, treated with dilute sodium bicarbonate (2 %) solution and followed with cold distilled water. The solid obtained was dried and recrystallised.

*Synthesis of 1-(4-(2,5-dichlorophenyl)-4H-1,3-thiazet-2-yl)-5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole (**9a**)* IR (KBr) ν max, cm^{-1}: imine (C=N) 2250. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.27–3.30 (dd, 1H, J_{AB} = 17.02, J_{Ax} = 5.04, C_4–H_A of pyrazole), 3.84–3.88 (dd, 1H, J_{Bx} = 11.04, C_4–H_B of pyrazole), 5.95–5.98 (dd, 1H, C_5–H_x of pyrazole), 6.32 (s, 1H, CH, thiazet), 6.72–7.64 (m, 11H, ArH) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 37.14, 62.33 (C_4, C_5 of pyrazole), 87.92 (S–C of thiazet), 122.35–154.13 (phenyl-C), 155.16 (C=N of pyrazole), 175.16 (C=N of thiazet) ppm. *m*/*e*: 407.3 [(M+2)$^+$], 405.03 (100.0 %), 475.03 (68.7 %), 474.04 (25.0 %), 477.03 (13.4 %).

*Synthesis of 5-(2,6-difluorophenyl)-3-phenyl-1-(4-phenyl-4H-1,3-thiazet-2-yl)-4,5-dihydro-1H-pyrazole (**9b**)* IR (KBr) ν max, cm^{-1}: imine(C=N) 2252. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.24–3.28 (dd, 1H, J_{AB} = 17.01, J_{Ax} = 5.11, C_4–H_A of pyrazole), 3.82–3.85 (dd, 1H, J_{Bx} = 11.04, C_4–H_B of pyrazole), 5.98–6.02 (dd, 1H, C_5–H_x of pyrazole), 6.32 (s, H, CH, thiazet), 6.72–7.64 (m, 13H, ArH) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 36.22, 61.54 (C_4, C_5 of pyrazole), 86.87 (S–C of thiazet), 125.5–157.13 (phenyl-C), 156.25 (C=N of pyrazole), 172.21 (C=N of thiazet) ppm.

*Synthesis of 1-(4-(4-chlorophenyl)-4H-1,3-thiazet-2-yl)-5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole (**9c**)* IR (KBr) *v* max, cm^{-1}: imine(C=N) 2255. ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.26–3.29 (dd, 1H, $J_{AB} = 17.01$, $J_{Ax} = 5.13$, C_4–H_A of pyrazole), 3.84–3.88 (dd, 1H, $J_{Bx} = 11.04$, C_4–H_B of pyrazole), 5.92–5.98 (dd, 1H, C_5–H_x of pyrazole), 6.51–7.81 (m, 12H, ArH), 6.28 (s,1H,CH, thiazet) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 114.5–167.77 (phenyl-C), 152.43 (C=N of pyrazole), 171.45 (C=N of thiazet), 36.22, 64.34 (C_4, C_5 of pyrazole), 85.82 (S–C of thiazet) ppm.

*Synthesis of 5-(2,6-difluorophenyl)-1-(4-(4-nitrophenyl)-4H-1,3-thiazet-2-yl)-3- phenyl-4,5-dihydro-1H-pyrazole (**9d**)* IR (KBr) *v* max, cm^{-1}: imine (C=N) 2257. ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.24–3.28 (dd, 1H, $J_{AB} = 17.02$, $J_{Ax} = 5.08$, C_4–H_A of pyrazole), 3.91–3.94 (dd, 1H, $J_{Bx} = 11.04$, C_4–H_B of pyrazole), 5.87–5.89 (dd, 1H, C_5–H_x of pyrazole), 6.12–7.81 (m, 12H, ArH), 6.24 (s,1H,CH, thiazet) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 113.1–162.37 (phenyl-C), 151.11 (C=N of pyrazole), 174.25 (C=N of thiazet), 35.12, 63.33 (C_4, C_5 of pyrazole), 82.45 (S–C of thiazet) ppm.

*Synthesis of 4-(4-(5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)-2H-1,3-thiazet-2-yl)phenol (**9e**)* IR (KBr) *v* max, cm^{-1}: imine(C=N) 2257. ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.22–3.25 (dd, 1H, J_{AB} 17.04, $J_{Ax} = 5.11$ C_4–H_A of pyrazole), 3.92–3.97 (dd, 1H, $J_{Bx} = 11.04$, C_4–H_B of pyrazole), 5.85–5.87 (dd, 1H, C_5–H_x of pyrazole), 6.12–7.75 (m, 12H, ArH), 6.24 (s, 1H, CH, thiazet), 2.35 (s, 3H, CH_3 of *p*-methylphenyl) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 114.1–171.25 (phenyl-C), 152.55 (C=N of pyrazole), 174.25 (C=N of thiazet), 35.12, 63.33 (C_4, C_5 of pyrazole), 24.3 (CH_3 of *p*-methyl phenyl), 81.45 (S–C of thiazet) ppm. *m/e*: 452.32 [$(M+2)^+$], 450.10 (100.0 %), 451.10 (25.9 %), 452.09 (4.5 %).

*Synthesis of 4-(4-(5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)-2H-1,3-thiazet-2-yl)phenol (**9f**)* IR (KBr) *v* max, cm^{-1}: imine(C=N) 2254. ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.24–3.29 (dd, 1H, J_{AB} 17.04, $J_{Ax} = 5.06$, C_4–H_A of pyrazole), 3.94–3.99 (dd, 1H, $J_{Bx} = 11.04$, C_4–H_B of pyrazole), 5.82–5.88 (dd, 1H, C_5–H_x of pyrazole), 6.15–7.78 (m, 12H, ArH), 5.02 (s, 1H, OH of *p*-hydroxy phenyl), 6.14 (s,1H,CH, thiazet) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 32.12, 61.34 (C_4, C_5 of pyrazole), 81.25(S–C of thiazet), 114.1–171.25 (phenyl-C), 152.55 (C=N of pyrazole), 172.41 (C=N of thiazet) ppm.

*Synthesis of 5-(2,6-difluorophenyl)-1-(4-(4-methoxyphenyl)-4H-1,3-thiazet-2-yl)-3-phenyl-4,5-dihydro-1H-pyrazole (**9g**)* IR (KBr) *v* max, cm^{-1}: imine(C=N) 2258. ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.28–3.32 (dd, 1H, J_{AB} 17.04, $J_{Ax} = 5.08$, C_4–H_A of pyrazole), 3.71 (methoxy phenyl OCH_3),3.98–4.02 (dd, 1H, J_{Bx} 11.04, C_4–H_B of pyrazole), 5.84–5.87 (dd, 1H, C_5–H_x of pyrazole), 6.05–7.71 (m, 12H, ArH), 6.22 (s, 1H, CH, thiazet) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 32.12, 61.34 (C_4, C_5 of pyrazole), 81.25(S–C of thiazet), 114.1–171.25 (phenyl-C), 152.55 (C=N of pyrazole), 172.41 (C=N of thiazet) ppm.

*Synthesis of 1-(4-(4-substituted thiazol-2-yl)-5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole derivative (**10a–g**)*

To a suspension of compound **4** (0.01 mol) in ethanol (20 mL), phenacyl bromide derivatives (0.01 mol) were added and heated at reflux for 1 h. After cooling, the product was filtered and recrystallized from an appropriate solvent.

*Synthesis of 5-(2,6-difluorophenyl)-3-phenyl-1-(4-phenylthiazol-2-yl)-4,5-dihydro-1H-pyrazole (**10a**)* IR (KBr) *v* max, cm^{-1}: (C=N) 1585, (C=C) 1510. ^1H NMR (DMSO-d6, 300 MHz) δ: 3.25–3.27 (dd, 1H, $J_{AB} = 17.05$, $J_{Ax} = 5.11$, C_4–H_A of pyrazole), 3.82–3.86 (dd, 1H, $J_{Bx} = 11.04$, C_4–H_B of pyrazole), 5.92–5.94 (dd, 1H, C_5–H_x of pyrazole), 6.94 (s, 1H, CH, thiazole), 6.82–7.71 (m, 13H, ArH) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 42.18, 62.11 (C_4, C_5 of pyrazole), 117.11–156.21 (phenyl-C), 157.54 (C=N, pyrazole), 104.25, 157.26, 164.85 (C_5, C_4, C_2 of thiazole) ppm. *m/e*: 419.26 [$(M+2)^+$], 417.11 (100.0 %), 418.11 (27.9 %), 419.11 (5.0 %).

*Synthesis of 3-(2-(5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)pyridine (**10b**)* IR (KBr) *v* max, cm^{-1}: (C=N) 1582, (C=C) 1513. ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.24–3.26 (dd, 1H, $J_{AB} = 17.08$, $J_{Ax} = 5.12$, C_4–H_A of pyrazole), 3.84–3.90 (dd, 1H, $J_{Bx} = 11.04$, C_4–H_B of pyrazole), 5.91–5.97 (dd, 1H, C_5–H_x of pyrazole), 6.92 (s, 1H, CH, thiazole), 6.88–7.85 (m, 12H, ArH) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 152.24 (C=N, pyrazole), 116.11–165.44 (phenyl-C), 105.14, 156.76, 165.71 (C_5, C_4, C_2 of thiazole), 45.11, 65.82 (C_4, C_5 of pyrazole) ppm.

*Synthesis of 1-(4-(4-chlorophenyl)thiazol-2-yl)-5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole (**10c**)* IR (KBr) *v* max, cm^{-1}: (C=N) 1581, (C=C) 1517. ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.25–3.28 (dd, 1H, $J_{AB} = 17.02$, $J_{Ax} = 5.12$, C_4–H_A of pyrazole), 3.82–3.86 (dd, 1H, $J_{Bx} = 11.04$, C_4–H_B of pyrazole), 5.92–5.96 (dd, 1H, C_5–H_x of pyrazole), 6.85 (s, 1H, CH, thiazole), 6.42–7.91 (m, 12H, ArH) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 44.55, 64.25 (C_4, C_5 of pyrazole), 104.71,154.25,162.52

(C_5, C_4, C_2 of thiazole), 116.11–167.44 (phenyl-C), 158.62 (C=N, pyrazole), 182.55 (C=O), 154.25 (C–O) ppm.

Synthesis of 5-(2,6-difluorophenyl)-3-phenyl-1-(4-p-tolylthiazol-2-yl)-4,5-dihydro-1H-pyrazole ***(10d)*** IR (KBr) ν max, cm^{-1}: (C=N) 1588, (C=C) 1514. ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.32 (s, 3H, CH_3 of *p*-methyl phenyl), 3.22–3.26 (dd, 1H, $J_{AB} = 17.01$, $J_{Ax} = 5.12$, C_4–H_A of pyrazole), 3.92–3.95 (dd, 1H, $J_{Bx} = 11.04$, C_4–H_B of pyrazole), 5.85–5.88 (dd, 1H, C_5–H_x of pyrazole), 6.28–7.92 (m,12H, ArH), 6.88 (s,1H,CH, thiazole), ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 24.3 (CH_3 of *p*-methyl phenyl), 43.55, 61.17 (C_4, C_5 of pyrazole), 104.85, 157.15, 161.62 (C_5, C_4, C_2 of thiazole), 115.11–162.44 (phenyl-C), 156.71 (C=N, pyrazole) ppm.

Synthesis of 4-(2-(5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl)thiazol-4-yl)phenol ***(10e)*** IR (KBr) ν max, cm^{-1}: (C=N) 1581, (C=C) 1508. ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.26–3.29 (dd, 1H, $J_{AB} = 17.01$, $J_{Ax} = 5.12$, C_4–H_A of pyrazole), 3.96–3.98 (dd, 1H, $J_{Bx} = 11.04$, C_4–H_B of pyrazole), 5.02 (s, 1H, OH of *p*-hydroxy phenyl), 5.86–5.89 (dd, 1H, C_5–H_x of pyrazole), 6.82 (s, 1H, CH, thiazole), 6.22–7.81 (m, 12H, ArH) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 43.55, 61.17 (C_4, C_5 of pyrazole), 104.94, 157.62, 161.74 (C_5, C_4, C_2 of thiazole), 115.11–165.54 (phenyl-C), 152.58 (C=N, pyrazole), 158.54 (C–O), 182.11 (C=O) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 43.55, 61.17 (C_4, C_5 of pyrazole), 104.94, 157.62, 161.74 (C_5, C_4, C_2 of thiazole), 115.11–165.54 (phenyl-C), 152.58 (C=N, pyrazole), 158.54 (C–O), 182.11 (C=O) ppm.

Synthesis of 5-(2,6-difluorophenyl)-1-(4-(4-nitrophenyl) thiazol-2-yl)-3-phenyl-4,5-dihydro-1H-pyrazole ***(10f)*** IR (KBr) ν max, cm^{-1}: (C=N) 1580, (C=C) 1507. ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.28–3.32 (dd, 1H, $J_{AB} = 17.04$, $J_{Ax} = 5.11$, C_4–H_A of pyrazole), 3.92–3.96 (dd, 1H, $J_{Bx} = 11.04$, C_4–H_B of pyrazole), 5.82–5.85 (dd, 1H, C_5–H_x of pyrazole), 6.54 (s, 1H, CH, thiazole), 6.24–7.57 (m, 12H, ArH) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 43.55, 61.17 (C_4, C_5 of pyrazole), 103.25, 158.55, 161.62 ($C_{5,4,2}$ of thiazole), 115.11–165.54 (phenyl-C), 159.25 (C=N, pyrazole) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 43.55, 61.17 (C_4, C_5 of pyrazole), 103.25, 158.55, 161.62 ($C_{5,4,2}$ of thiazole), 115.11–165.54 (phenyl-C), 159.25 (C=N, pyrazole) ppm.

Synthesis of 1-(4-(4-bromophenyl)thiazol-2-yl)-5-(2,6-difluorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole ***(10g)*** IR (KBr) ν max, cm^{-1}: (C=N) 1581, (C=C) 1516. ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.24–3.29 (dd, 1H, $J_{AB} = 17.04$, $J_{Ax} = 5.11$, C_4–H_A of pyrazole), 3.94–3.99 (dd, 1H, $J_{Bx} = 11.04$, C_4–H_B of pyrazole), 5.81–5.86 (dd, 1H, C_5–H_x of pyrazole), 6.51 (s, 1H, CH, thiazole), 6.14–7.87 (m, 12H, ArH) ppm. ^{13}C NMR (DMSO-d_6, 300 MHz) δ: 43.55, 61.17 (C_4, C_5 of pyrazole), 103.43, 159.61, 164.93 (C_5, C_4, C_2 of thiazole), 115.11–165.54 (phenyl-C), 158.26(C=N, pyrazole) ppm.

Biological protocol

Antimicrobial activity

The antimicrobial susceptibility testing was performed in vitro by broth micro dilution method (Hassan *et al.*, 1993; Khalil *et al.*, 1993). The MIC determination of the synthesized 4,5-dihydropyrazole derivatives (**4**, **5**, **6**, **7a**–**h**, **8**, **9a**–**g**, and **10a**–**g**) was carried out in side-by-side comparison with ciprofloxacin and norfloxacin against Gram-positive (*S. aureus*, *S. faecalis, B. subtilis*) and Gram-negative (*K. penumoniae*, *E. coli. P. aeruginosa*) bacteria. The antifungal activity was assayed against yeasts (*C. tropicalis*, *S. cerevisiae*) and moulds (*A. niger*). The minimal inhibitory concentrations (MIC, μg/mL) were defined as the lowest concentrations of compound that completely inhibited the growth of each strain. Test compounds (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL) then diluted in culture medium (Mueller-Hinton broth for bacteria and Sabouraud liquid medium for fungi), further progressive dilutions to obtain final concentrations of 1, 2, 4, 8, 16, 31.25, 62.5, 125, 250 and 500 μg/mL. DMSO never exceeded 1 % v/v. The tubes were inoculated with 10^5 cfu/mL (colony forming unit/mL) and incubated at 37 °C for 24 h. The growth control consisting of media (positive control) and media with DMSO (negative control) at the same dilutions as used in the experiments were employed.

Anticancer activity

The synthesized 4,5-dihydropyrazole derivatives (**4**, **5**, **6**, **7a**–**h**, **8**, **9a**–**g** and **10a**–**g**) were tested in vitro for their cytotoxic properties against tumor cell lines panel consisted of Hela (human cervix carcinoma cell line), A549 (Human lung adenocarcinoma cell line), MCF-7 (human breast adenocarcinoma cell line), A2780 (human ovarian cancer cell line), and BGC-823 (human gastric cancer cell line) by using MTT assay Mosmann's method. The MTT assay is based on the reduction of the soluble MTT (0.5 mg/mL, 100 μL), into a blue-purple formazan product, mainly by mitochondrial reductase activity inside living cells (Mosmann, 1983).

The cells used in cytotoxicity assay were cultured in RPMI 1640 medium supplemented with 10 % fetal calf serum, penicillin, and streptomycin at 37 °C and humidified at 5 % CO_2. Briefly cells were placed on 96-well plates at 100 μL total volume with density of 1–2.5 × 10^4 cells/mL and were allowed to adhere for 24 h before treatment with tested drugs in DMSO solution (10^{-5}, 10^{-6}, 10^{-7} mol/L final concentration). Triplicate wells were treated with media and agents. Cell viability was assayed after 96 h of continuous drug exposure with a tetrazolium compound. The supernant medium was removed, and 150 μL of DMSO solution was added to each well. The plates were gently agitated using mechanical plate mixer until the color reaction was uniform and the OD570 was determined using microplate reader. The 50 % inhibitory concentration (IC50) was defined as the concentration that reduced the absorbance of the untreated wells by 50 % of vehicle in the MTT assay. Assays were performed in triplicate on three independent experiments. The results had good reproducibility between replicate wells with standard errors below 10 %.

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