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Short communication

Poly(ethylene glycol) (PEG-400) as an alternative reaction solvent for the synthesis of some new 1-(4-(4′-chlorophenyl)-2-thiazolyl)-3-aryl-5-(2-butyl-4-chloro-1*H*-imidazol-5yl)-2-pyrazolines and their *in vitro* antimicrobial evaluation

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ABSTRACT

Several 1-(4-(4′-chlorophenyl)-2-thiazolyl)-3-aryl-5-(2-butyl-4-chloro-1*H*-imidazol-5yl)-2-pyrazoline derivatives were prepared by the base catalyzed treatment of appropriate chalcones with 4-(4′-chlorophenyl)-2-hydrazino-thiazole in poly (ethylene glycol) (PEG-400) as an alternative reaction solvent. All the synthesized compounds were tested for their antimicrobial activities against *Escherichia coli* (MTCC 2939), *Salmonella typhi* (MTCC 98), *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 441), *Aspergillus niger* (MTCC 281), *Trichoderma viridae* (MTCC 167), *Penicillium chrysogenum* (MTCC 160), *Fusarium moniliforme* (MTCC 156) and *Candida albicans* (MTCC 183). Most of the compounds showed potent antibacterial and antifungal activity.

1. Introduction

There has been significant increase in the frequency of systematic fungal infection in humans. Patients undergoing organ transplants, anticancer chemotherapy, or long treatment with antimicrobial agents, and patients with AIDS, are immunosuppressed and very susceptible to life-threatening systematic fungal infections like Candidiasis, Cryptococcosis, and Aspergillosis. Resistance to antibacterial agents is a significant problem since last few decades [1–3]. In addition, primary and opportunistic fungal infections continue to increase rapidly because of the increased number of immunocompromised patients (AIDS, cancer and transplants). Several reviews have appeared illustrating the problems encountered by today's infectious disease clinicians [4,5].

To overcome this rapid development of drug resistance, new agents should preferably consist of chemical characteristics that clearly differ from those of existing agents. In the process of drug designing an essential component of the search for new leads is the synthesis of novel molecules, which are biologically active by the virtue of the presence of critical structural features. Electron-rich nitrogen heterocycles play an important role in diverse biological activities. Introducing a pyrazolidinone ring [6,7] in place of the β-lactam ring (in penicillins and cephalosporins [8] results in enhanced activity). Second nitrogen in the five-membered ring also influences the antibacterial or pharmacokinetic properties [9,10]. Pyrazoline derivatives have also been reported in the literature to exhibit various pharmacological activities such as anti-inflammatory [11], antihypertensive [12], and antimicrobial [13–15]. On the other hand, sulfur and/or nitrogen heterocycles having pharmaceutical activities are widely occur in nature in the form of alkaloids, vitamins, pigments and as a constituents of plant and animal cells. Penicillins containing a thiazole ring system (thiazolidine) [16] are also important naturally occurring products. Thiazoles and their derivatives are found to possess various biological activities such as antituberculosis [17], anti-HIV [18].

2. Chemistry

The synthetic pathway of the compounds is outlined in the scheme 1 and 2. The starting 4-(4′-chlorophenyl)-2-hydrazino-thiazole (**I**) was prepared by the treatment of 4-chloro-α-haloketone with thiosemicarbazide in PEG-400 (10 mL) at 40 °C, while

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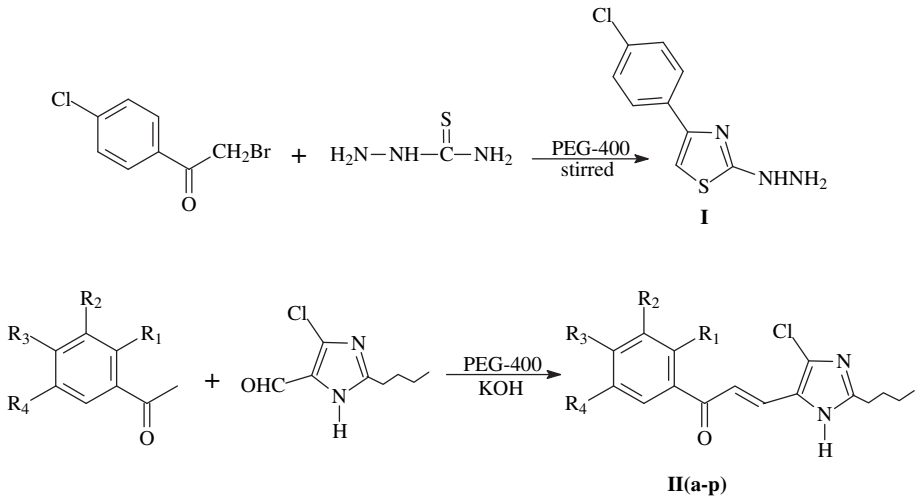


Scheme 1. Synthesis of 2-hydrazino-thiazole (**I**) and Chalcones (**II**).

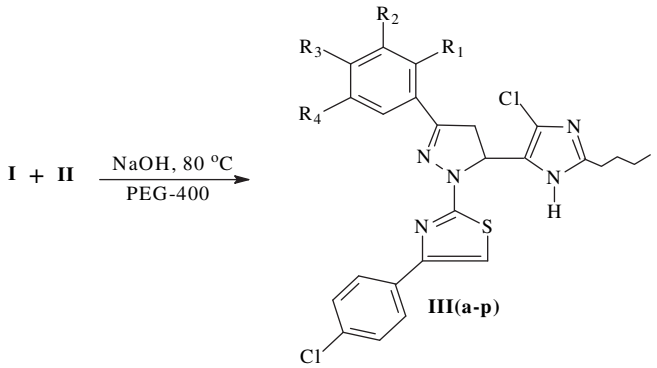
the novel α,β-unsaturated carbonyl compounds (**IIa**–**p**) were prepared by Claisen -Schmidt condensation by reacting substituted acetophenones and 2-butyl-4-chloro-5-formyl-imidazole (BCFI) in PEG-400 at 40 °C for 1 h (Scheme 1). Finally, the synthesis of 1-thiazolyl-2-pyrazolines (**IIIa**–**p**) was attempted by the reacting 4-(4′-chlorophenyl)-2-hydrazino-thiazole with α,β-unsaturated carbonyl compounds (chalcones) using NaOH in PEG-400 under mild reaction condition (Scheme 2).

3. Biology

The antimicrobial activities of the synthesized compounds (**IIIa**–**p**) were determined by agar diffusion method as recommended by the National Committee for Clinical Laboratory Standards, (NCCLS) [19–21]. The compounds were evaluated for antimicrobial activity against bacteria *viz. Escherichia coli* (MTCC 2939), *Salmonella typhi* (MTCC 98), *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 441) and antifungal activity against various fungi *viz. Aspergillus niger* (MTCC 281), *Trichoderma viridae* (MTCC 167), *Penicillium chrysogenum* (MTCC 160), *Candida albicans* (MTCC 183) and *Fusarium moniliforme* (MTCC 156). The antibiotic Tetracycline (25 μg/mL) and Nystatin (25 μg/mL) are used as reference antibacterial and antifungal substances, respectively for comparison. Dimethyl sulphoxide (1%, DMSO) was used a control.

The culture strains of bacteria were maintained on nutrient agar slant at 37 ± 0.5 °C for 24 h. The antibacterial activity was evaluated using nutrient agar plate seeded with 0.1 mL of respective bacterial culture strain suspension prepared in sterile saline (0.85%) of 10^5 CFU/mL dilutions. The wells of 6 mm diameter were filled with 0.1 mL of target compound dilution ranging from 25 to 250 μg/mL separately for each bacterial strain. All the plates were incubated at 37 ± 0.5 °C for 24 h. Zone of inhibition and minimum inhibitory concentrations (*MICs*) were noted. The results of antibacterial studies are given in Table 2.

For antifungal activity, all the culture strains of fungi maintained on potato dextrose agar (PDA) slant at 27 ± 0.2 °C for 24–48 h, till sporulation. Spore of strains were transferred in to 5 mL of sterile distilled water containing 1% Tween-80 (to suspend the spore properly). The spores were counted by haemocytometer (10^6 CFU/mL). Sterile PDA plate was prepared containing 2% agar; 0.1 mL of each fungal spore suspension was spread on each plate and incubated at 27 ± 0.2 °C for 12 h. After incubation well prepared using sterile cork borer and each agar well was filled with 0.1 mL compound solution of concentrations 25–250 μg/mL separately to get minimum inhibitory concentration (*MIC*) value of 1-thiazolyl-2-pyrazolines. The plates were kept in refrigerator for 20 min for diffusion and then incubated at 27 ± 0.2 °C for 24–28 h. Zone of inhibition and minimum inhibitory concentrations (*MICs*) were noted. The results of antifungal studies are given in Table 3.



Scheme 2. Synthesis of target molecules (1-thiazolyl-2-pyrazolines) (**III**).

4. Results and discussion

Prompted by the above mentioned biological properties of pyrazolines, it was contemplated to synthesize some new series of

Table 1
Synthesis of some new l-thiazolyl-2-pyrazolines using PEG-400.

Product	R_1	R_2	R_3	R_4	Time[a]	Yield(%)[b]	M.P.(°C)
IIIa	OH	H	H	Cl	35	90	134
IIIb	H	H	Cl	H	20	92	148
IIIc	OH	I	H	Cl	35	88	162
IIId	OH	Br	H	Cl	25	90	125
IIIe	OH	H	Me	Cl	30	89	118
IIIf	OH	I	Me	Cl	30	88	152
IIIg	OH	Br	Me	Cl	35	88	122
IIIh	H	H	NH_2	H	30	89	138
IIIi	H	H	OMe	H	30	90	145
IIIJ	OH	I	H	Me	35	88	156
IIIk	OH	Br	H	Me	30	89	135
IIIl	OH	H	H	Me	40	88	128
IIIm	OH	H	H	H	30	87	115
IIIn	OH	I	H	I	35	88	168
IIIo	OH	Br	H	Br	25	90	154
IIIp	OH	Cl	H	Cl	30	91	140

[a] Time in minutes.
[b] Pure isolated yields of products.

Table 2
Antibacterial activities of 1-thiazolyl-2-pyrazolines (**IIIa–p**).

Compound	Ec	St	Sa	Bs
IIIa	18 (25)	12 (50)	16 (25)	18 (25)
IIIb	16 (25)	10 (50)	12 (50)	14 (50)
IIIc	20 (25)	16 (25)	18 (25)	21 (25)
IIId	21 (25)	18 (25)	16 (25)	15 (25)
IIIe	14 (50)	09 (50)	12 (50)	–
IIIf	18 (25)	16 (25)	14 (50)	19 (25)
IIIg	15 (25)	12 (50)	11 (50)	18 (25)
IIIh	12 (50)	–	10 (50)	08 (50)
IIIi	16 (25)	14 (50)	18 (25)	16 (25)
IIIj	14 (50)	15 (25)	15 (25)	12 (50)
IIIk	16 (25)	12 (50)	12 (50)	14 (50)
IIIl	11 (50)	–	–	12 (50)
IIm	15 (50)	10 (50)	13 (50)	16 (50)
IIIn	20 (25)	18 (25)	16 (25)	20 (25)
IIIo	22 (25)	19 (25)	18 (25)	21 (25)
IIIp	21 (25)	18 (25)	16 (25)	22 (25)
Tetracycline	30 (25)	28 (25)	30 (25)	32 (25)

Zone of inhibition measured in mm; MIC values (μg/mL) are given in brackets.
Ec-*Escherichia coli*; St-*Salmonella typhi*.
Sa-*Staphylococcus aureus*; Bs-*Bacillis subtilis*.
"–" Indicates the concentration > 100 μg/mL.

thiazolyl pyrazolines under the frame of 'green chemistry'. In recent years, poly (ethylene glycol) prompted reactions [22–26] have attracted the attention of organic chemists due to their solvating ability and aptitude to act as a phase transfer catalyst, negligible vapor pressure, easy recyclability, ease of work-up, eco-friendly nature and economical cost. PEG is non-toxic, non-halogenated, inexpensive potentially recyclable and water soluble which facilitate its removal from reaction product. To best of our knowledge, there are no earlier reports for the synthesis of 1-thiazolyl-2-pyrazolines using poly (ethylene glycol) (PEG-400) as green reaction medium.

In this report, we wish to describe a mild and efficient method for the synthesis of 1-(4-(4′-chlorophenyl)-2-thiazolyl)-3-aryl-5-(2-butyl-4-chloro-1*H*-imidazol-5yl)-2-pyrazolines using poly (ethylene glycol) (PEG-400) as an alternative reaction solvent. The target molecules were attempted by the treatment of 4-(4′-chlorophenyl)-2-hydrazino-thiazole with α,β-unsaturated carbonyl compounds (chalcones) using NaOH in PEG-400 under mild reaction condition.

Table 3
Antifungal activities of 1-thiazolyl-2-pyrazolines (**IIIa–p**).

Compound	An	Tv	Pc	Ca	Fm
IIIa	16 (25)	15 (25)	12 (25)	16 (25)	15 (25)
IIIb	12 (50)	11 (50)	10 (50)	14 (25)	11 (50)
IIIc	15 (25)	16 (25)	14 (25)	15 (25)	12 (25)
IIId	18 (25)	15 (25)	16 (25)	16 (25)	10 (50)
IIIe	12 (50)	14 (25)	13 (25)	–	11(50)
IIIf	14 (25)	12 (25)	15 (25)	13 (25)	14 (25)
IIIg	15 (25)	16 (50)	09 (50)	12 (50)	15 (25)
IIIh	14 (25)	12 (50)	10 (50)	–	–
IIIi	16 (25)	14 (25)	12 (50)	14 (25)	12 (25)
IIIj	14 (25)	14 (25)	12 (25)	11 (50)	10 (50)
IIIk	10 (50)	14 (25)	13 (50)	12 (25)	09 (50)
IIIl	12 (50)	08 (50)	–	10 (50)	11 (50)
IIm	14 (25)	16 (25)	14 (25)	15 (25)	13 (25)
IIIn	19 (25)	20 (25)	16 (25)	18 (25)	16 (25)
IIIo	20 (25)	18 (25)	18 (25)	20 (25)	14 (25)
IIIp	19 (25)	18 (25)	18 (25)	16 (25)	15 (25)
Nystatin	18 (25)	18 (25)	16 (25)	18 (25)	16 (25)

Zone of inhibition measured in mm; MIC values (μg/mL) are given in brackets.
An-*Aspergillus niger*; Tv-*Trichoderma virdea*; Ca-*Candida albicans*.
Pc-*Penicillium chrysogenum*; Fm-*Fusarium moniliforme*.
"–" Indicates the concentration > 100 μg/mL.

All the structures of products were appropriately established by spectroscopic data and analytical methods. The IR spectra of all the products showed disappearance of band at 1640–1655 cm^{-1} due to >C═O of chalcones. The ^1H NMR spectra of the compounds, the CH_2 protons of the pyrazoline ring resonated as a pair of doublets of doublets at δ 3.34–3.62 ppm (H_A), δ 3.92–4.21 ppm (H_B). The –CH (H_x) proton appeared as a doublet of doublets at δ 5.23–5.71 ppm due to vicinal coupling with the two magnetically non-equivalent protons of the methylene group at position 4 of the pyrazoline ring ($J_{AB} = 17.7–18.3$ Hz, $J_{Ax} = 8.1–8.4$, $J_{Bx} = 12.0–12.3$). The -5H of thiazole was observed as singlet in the range of 6.95–7.15 ppm. Phenolic proton appeared as a singlet near δ 11.0–13.0 due to the hydrogen bonding, while other aromatic and aliphatic protons were observed at excepted regions. ^{13}C NMR data of compound **IIIe** exhibited the chemical shift values of the carbon atoms at δ 43 ppm (C-4), δ 66 ppm (C-5) and δ 154 ppm (C-3) of the 2-pyrazoline ring. The chemical shift value at δ 168 ppm corroborates the C-2 of the thiazole. The mass spectra (EIMS) of compounds were also in agreement with their molecular formula.

The results of *in vitro* antibacterial activities of compounds (**IIIa–p**) against various bacterial strains are summarized in Table 2. It has been observed that some of compounds exhibited interesting antibacterial activities. Compounds **IIIa**, **IIIb**, **IIIf**, **IIIg**, **IIIi** and **IIIk** showed effective activity against *E. coli*, and compounds **IIIa**, **IIIf**, **IIIg** and **IIIi** were displayed a good zone of inhibition against *B. subtilis*. Compounds **IIIb**, **IIIg** and **IIIk** displayed a slightly active towards *S. typhi* and *S. aureus*. Compounds **IIIe**, **IIIl**, **IIIh**, and **IIIm** were displayed less active against all tested bacteria. On the other hand, it was found that compounds **IIIc**, **IIId**, **IIIn**, **IIIo** and **IIIp** were showed stronger inhibitory activity against all bacteria than other compounds.

The results of antifungal activities of synthesized compounds (**IIIa–p**) were summarized in Table 3. Most of the compounds were showed a significant level of activity at concentration 25 μg/mL, in comparison with standard antifungal. Compounds **IIIa**, **IIIc**, **IIId**, and **IIIi** were showed a good inhibitory activity against *A. niger* and the compounds **IIIn**, **IIIo**, and **IIIp** exhibited greater antifungal activity than standard Nystatin against *A. niger*. Compounds **IIIa**, **IIIc**, **IIId**, **IIIf**, **IIIi**, **IIIj**, **IIIk** and **IIIm** were showed good activity against *T. viridae*. Compounds **IIIo** and **IIIp** were displayed similar level of activity and **IIIn** showed higher activity than standard against *T. viridae*. Compounds **IIIa**, **IIIc**, **IIIe**, **IIIj** and **IIIm** were showed moderate activity towards *P. chrysogenum*. Compounds **IIId**, **IIIn** were showed equivalent activity and **IIIo**, **IIIp** were displayed stronger activity than standard antifungal drug against *P. chrysogenum*. Compounds **IIIa**, **IIIb**, **IIIc**, **IIId**, **IIIf**, **IIIi**, **IIIk**, **IIIm** and **IIIn** were more sensitive towards *C. albicans*. Compounds **IIIn** showed similar level of activity and **IIIo** displayed higher activity than standard drug against *C. albicans*. Compounds **IIIa**, **IIIc**, **IIIf**, **IIIg**, **IIIi**, **IIIm**, **IIIo** and **IIIp** were exhibited good zone of inhibition against *F. moniliforme* as compared with standard antibiotic. Only the compound **IIIn** showed similar level of activity against *F. moniliforme*. From the studies of antifungal activity, it was observed that compounds **IIIn**, **IIIo** and **IIIp** were exhibited more potent activity against all fungal strains than other compounds. Considering the results obtained from antifungal and antibacterial tests together, it is noteworthy to mention that tested compounds are more active towards fungi than bacteria.

5. Conclusion

In summary, we have developed a novel, efficient and environmentally benign methodology towards the synthesis of 1-(4-(4′-chlorophenyl)-2-thiazolyl)-3-aryl-5-(2-butyl-4-chloro-1*H*-

imidazol-5yl)-2-pyrazolines by the reaction of chalcones with 2-hydrazino-thiazole in PEG-400 as an alternative reaction solvent. Pyrazolines carrying 2-hydroxy-3-iodo-5-chloro-phenyl (**IIIc**), 2-hydroxy-3-bromo-5-chloro-phenyl (**IIId**), 2-hydroxy-3, 5-di-iodo-phenyl (**IIIn**), 2-hydroxy-3, 5-dibromo-phenyl (**IIIo**), and 2-hydroxy-3, 5-dichloro-phenyl (**IIIp**) were exhibited stronger antifungal and antibacterial activity. The substitution of hydroxyl group in position 2 and presence of halo groups in 3 and 5 positions emerged as active in both antibacterial and antifungal screening. Hence, it is concluded that there is enough scope for further study in the developing these as good lead compounds.

6. Experimental

Melting points were determined by in an open capillary method and are uncorrected. The chemicals and solvents used for laboratory grade and were purified. IR spectra were recorded (in KBr pallets) on Shimadzu spectrophotometer. ^{1}H NMR spectra were recorded (in DMSO-d_6) on Avance-300 MHz spectrometer using TMS as an internal standard. The mass were recorded on EI-Shimadzu GC–MS spectrometer. Elemental analyses were performed on a Carlo Erba 106 Perkin–Elmer model 240 analyzer.

6.1. General procedure for the synthesis of 4-(4′-chlorophenyl)-2-hydrazino-thiazole (*I*)

A mixture of 4-chloro-α-halo-ketone (5 mmol), thiosemicarbazide (5 mmol), and was stirred in PEG-400 (10 mL) at 40 °C for 20 min. After completion (TLC), the reaction mixture was extracted with diethyl ether (2 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was recrystallized from 20% aqueous acetic acid to afford to the pure product.

(***I***): M.P. 158 °C; Yield, 88%; IR (KBr, cm^{-1}): 1586, 1610; ^{1}H NMR (DMSO-d_6, δ ppm): 6.95 (s, 1H, -5H of thiazole), 7.22 (s, 2H, NH_2), 7.52 (d, 1H, J = 9 Hz), 7.71 (d, 1H, J = 9 Hz), 8.26 (s, 1H, NH); EIMS (m/z): 224 (M^+).

6.2. General procedure for synthesis of 1-(Substituted phenyl)-3-(2-butyl-4-chloro-1H-imidazol-5yl)-2-propen-1-ones (*IIa–p*)

An equimolar mixture of substituted acetophenone (1 mmol), 2-butyl-4-chloro-5-formyl-imidazole (1 mmol) and KOH (2 mmol) was stirred in PEG-400 (15 mL) at 40 °C for 1 h. After completion of the reaction (monitored by TLC), the crude mixture was worked up in ice cold water (100 mL). Product separated out was filtered and processed out. Filtrate was evaporated to remove water leaving PEG behind. The same PEG was utilized to synthesize further chalcones.

6.3. 1-(4-chlorophenyl)-3-(2-butyl-4-chloro-1H-imidazol-5yl)-2-propen-1-one (*IIb*)

M.P. 115 °C; Yield, 91%; IR (KBr, cm^{-1}): 1598 (–C = N), 1651 (>C = O), 3335 (–NH); ^{1}H NMR (DMSO-d_6, δ ppm): 0.93 (t, J = 7.2 Hz, 3H, $-CH_3$), 1.32 (m, 2H, $-CH_2-$), 1.64 (m, 2H, $-CH_2-$), 2.72 (t, J = 7.5 Hz, 2H, $-CH_2$), 7.12–7.98 (m, 6H, Ar–H + CH = CH), 8.19 (s, 1H, –NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, δ ppm): 14, 22, 29, 30, 117, 122, 128, 129 (2 × C), 130 (2 × C) 134, 136, 138, 152, 187; EIMS (m/z): 322 [M^+], 324 [M + 2] (14), 326 [M + 4] (8), 287 [100%]; Anal. Calcd. For $C_{16}H_{16}ON_2Cl_2$: C, 59.62; H, 4.96; N, 8.69%. Found: C, 59.54; H, 4.81; N, 8.74%

6.4. Typical procedure for the synthesis of 1-(4-(4′-chlorophenyl)-2-thiazolyl)-3-aryl-5-(2-butyl-4-chloro-1H- imidazol-5yl)-2-pyrazolines (*IIIa–p*)

A mixture of chalcone **II** (5 mmol), 2-hydrazino thiazole **I** (5 mmol), NaOH (10 mmol) and PEG-400 (20 mL) was stirred at room temperature for 5 min and then temperature raised to 80 °C for the period as shown in Table 1. After completion of reaction (TLC), the reaction mixture was cooled and poured in ice cold water (25 mL). The obtained solid product was filtered and washed with 2 × 5 mL water and recrystallized by aqueous acetic acid to give pure product **III**. PEG-400 was recovered from water by direct distillation and reused for second run by charging the same substrates.

6.5. 1-(4-(4′-chlorophenyl)-2-thiazolyl)-3-(5-chloro-2-hydroxyphenyl)-5-(2-butyl-4-chloro-1H-imidazol-5yl)-2-pyrazoline (*IIIa*)

IR (KBr, cm^{-1}): 1602 (–C=N), 3126 (–OH), 3336 (–NH); ^{1}H NMR (DMSO-d_6, δ ppm): 0.91 (t, J = 7.2 Hz, 3H, $-CH_3$), 1.31 (m, 2H, $-CH_2-$), 1.65 (m, 2H, $-CH_2-$), 2.75 (t, J = 7.5 Hz, 2H, $-CH_2$), 3.43 (dd, 1H, J = 17.7, 8.1 Hz), 4.04 (dd, 1H, J = 17.7, 12.0 Hz,), 5.54 (dd, 1H, J = 12.0, 8.1 Hz, -H_x), 7.05 (s, 1H, -5H of thiazole), 7.28–8.16 (m, 7H, Ar–H), 8.21 (s, 1H, –NH, D_2O exchangeable), 12.46 (s, 1H, –OH, D_2O exchangeable); EIMS (m/z): 546 (M^+), 548 (M + 2, 30), 550 (M + 4, 12), 552 (M + 6, 4); Anal. Calcd for $C_{25}H_{22}ON_5SCl_3$: C, 54.92; H, 4.02; N, 12.80%. Found: C, 54.78; H, 4.14; N, 12.68%

6.6. 1-(4-(4′-chlorophenyl)-2-thiazolyl)-3-(4-chlorophenyl)-5-(2-butyl-4-chloro-1H-imidazol-5yl)-2-pyrazoline (*IIIb*)

IR (KBr, cm^{-1}): 1595 (–C=N), 3333 (–NH); ^{1}H NMR (DMSO-d_6, δ ppm): 0.91 (t, J = 7.2 Hz, 3H, $-CH_3$), 1.32 (m, 2H, $-CH_2-$), 1.65 (m, 2H, $-CH_2-$), 2.76 (t, J = 7.5 Hz, 2H, $-CH_2$), 3.41 (dd, 1H, J = 17.7, 8.1 Hz), 4.08 (dd, 1H, J = 17.7, 12.0 Hz), 5.51 (dd, 1H, J = 12.3, 8.1 Hz), 7.02 (s, 1H, -5H of thiazole), 7.26–8.15 (m, 8H, Ar–H), 8.22 (s, 1H, –NH, D_2O exchangeable); EIMS (m/z): 531(M^+), 287 (100%); Anal. Calcd. For $C_{25}H_{22}N_5SCl_3$: C, 56.58; H, 4.14; N, 13.19%. Found: C, 56.68; H, 4.21; N, 13.05%

6.7. 1-(4-(4′-chlorophenyl)-2-thiazolyl)-3-(5-chloro-2-hydroxy-3-iodophenyl)-5-(2-butyl-4-chloro-1H-imidazol-5yl)-2-pyrazoline (*IIIc*)

IR (KBr, cm^{-1}): 1605 (–C=N), 3158 (–OH), 3331 (–NH); ^{1}H NMR (DMSO-d_6, δ ppm): 0.93 (t, J = 7.2 Hz, 3H, $-CH_3$), 1.31 (m, 2H, $-CH_2-$), 1.65 (m, 2H, $-CH_2-$), δ 2.74 (t, J = 7.8 Hz, 2H, $-CH_2$), 3.48 (dd, 1H, J = 18.0, 8.1 Hz), 4.10 (dd, 1H, J = 18.0, 12.0 Hz), 5.58 (dd, 1H, J = 12.0, 8.1 Hz), δ 7.05 (s, 1H, -5H of thiazole), 7.23–8.16 (m, 6H, Ar–H), 8.20 (s, 1H, –NH, D_2O exchangeable), 12.51 (s, 1H, –OH, D_2O exchangeable); EIMS (m/z): 672 (M^+), 674 (M + 2, 44), 676 (M + 4, 16), 678 (M + 6, 5); Anal. Calcd. For $C_{25}H_{21}ON_5SCl_3I$: C, 44.64; H, 3.12; N, 10.40%. Found: C, 44.55; H, 3.15; N, 10.29%

6.8. 1-(4-(4′-chlorophenyl)-2-thiazolyl)-3-(3-bromo-5-chloro-2-hydroxyphenyl)-5-(2-butyl-4-chloro-1H-imidazol-5yl)-2-pyrazoline (*IIId*)

IR (KBr, cm^{-1}): 1604 (–C=N), 3142 (–OH), 3330 (–NH); ^{1}H NMR (DMSO-d_6, δ ppm): 0.91 (t, J = 7.5 Hz, 3H, $-CH_3$), 1.30 (m, 2H, $-CH_2-$), 1.65 (m, 2H, $-CH_2-$), δ 2.75 (t, J = 7.5 Hz, 2H, $-CH_2$), 3.46 (dd, 1H, J = 18.3, 8.4 Hz), 4.08 (dd, 1H, J = 18.3, 12.3 Hz), 5.51 (dd, 1H, J = 12.3, 8.4 Hz), 7.08 (s, 1H, -5H of thiazole), 7.26–8.18 (m, 6H, Ar–H), 8.21 (s, 1H, –NH, D_2O exchangeable), 12.38 (s, 1H, –OH, D_2O exchangeable);

EIMS (*m*/*z*): 625 (M^+), 627 (M + 2, 22), 629 (M + 4, 12), 631 (M + 6, 3); Anal. Calcd. For $C_{25}H_{21}ON_5SCl_3Br$: C, 47.99; H, 3.35; N, 11.19%. Found: C, 47.86; H, 3.41; N, 11.28%

*6.9. 1-(4-(4′-chlorophenyl)-2-thiazolyl)-3-(5-chloro-2-hydroxy-4-methylphenyl)-5-(2-butyl-4-chloro-1H-imidazol-5yl)-2-pyrazoline (**IIIe**)*

IR (KBr, cm^{-1}): 1597 (–C═N), 3118 (–OH), 3335 (–NH); ^1H NMR (DMSO-d_6, δ ppm): 0.92 (t, *J* = 7.5 Hz, 3H, $–CH_3$), 1.32 (m, 2H, $–CH_2–$), 1.65 (m, 2H, $–CH_2–$), 2.34 (s, 3H, CH_3), 2.76 (t, *J* = 7.8 Hz, 2H, $–CH_2$), 3.42 (dd, 1H, *J* = 18.3, 8.1 Hz), 4.05 (dd, 1H, *J* = 18.3, 12.3 Hz), 5.56 (dd, 1H, *J* = 12.3, 8.1 Hz), 7.06 (s, 1H, -5H of thiazole), 7.24–8.16 (m, 6H, Ar–H), 8.21 (s, 1H, –NH, D_2O exchangeable), 12.31 (s, 1H, –OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, δ ppm): 14, 16, 22, 28, 30, 43, 66, 113, 116, 122, 123, 124, 126, 128 (2 × C), 129 (2 × C), 130, 131, 132, 133, 138, 140, 142, 154, 168; EIMS (*m*/*z*): 559 (M^+), 561 (M + 2, 89), 563 (M + 4, 86), 565 (M + 6, 8), 317 (100); Anal. Calcd. For $C_{26}H_{24}ON_5SCl_3$: C, 55.69; H, 4.28; N, 12.48%. Found: C, 55.65; H, 4.18; N, 12.55%

*6.10. 1-(4-(4′-chlorophenyl)-2-thiazolyl)-3-(3-bromo-5-chloro-2-hydroxy-4-methylphenyl)-5-(2-butyl-4-chloro-1H-imidazol-5yl)-2-pyrazoline (**IIIg**)*

IR (KBr, cm^{-1}): 1599 (–C═N), 3138 (–OH), 3338 (–NH); ^1H NMR (DMSO-d_6, δ ppm): 0.91 (t, *J* = 7.2 Hz, 3H, $–CH_3$), 1.31 (m, 2H, $–CH_2–$), 1.65 (m, 2H, $–CH_2–$), 2.36 (s, 3H, CH_3), 2.75 (t, *J* = 7.5 Hz, 2H, $–CH_2$), 3.51 (dd, 1H, *J* = 18.0, 8.1 Hz), 4.12 (dd, 1H, *J* = 18.0, 12.0 Hz), 5.58 (dd, 1H, *J* = 12.0, 8.1 Hz), 7.08 (s, 1H, -5H of thiazole), 7.23–8.15 (m, 5H, Ar–H), 8.21 (s, 1H, –NH, D_2O exchangeable), 12.48 (s, 1H, –OH, D_2O exchangeable); EIMS (*m*/*z*): 639 (M^+), 641 (M + 2, 25), 643 (M + 4, 18), 645 (M + 6, 5) Anal. Calcd. For $C_{26}H_{23}ON_5SCl_3Br$: C, 48.82; H, 3.59; N, 10.94%. Found: C, 48.74; H, 3.68; N, 10.82%

*6.11. 1-(4-(4′-chlorophenyl)-2-thiazolyl)-3-(4-aminophenyl)-5-(2-butyl-4-chloro-1H-imidazol-5yl)-2-pyrazoline (**IIIh**)*

IR (KBr, cm^{-1}): 1602 (–C═N), 3331 (–NH); ^1H NMR (DMSO-d_6, δ ppm): 0.91 (t, *J* = 7.5 Hz, 3H, $–CH_3$), 1.31 (m, 2H, $–CH_2–$), 1.65 (m, 2H, $–CH_2–$), 2.75 (t, *J* = 7.5 Hz, 2H, $–CH_2$), 3.46 (dd, 1H, *J* = 17.7, 8.1 Hz), 4.11 (dd, 1H, *J* = 17.7, 8.1 Hz), 5.56 (dd, 1H, *J* = 12.0, 8.1 Hz), 7.05 (s, 1H, -5H of thiazole), 7.23–8.15 (m, 8H, Ar–H), 8.20 (s, 1H, –NH, D_2O exchangeable); EIMS (*m*/*z*): 511 (M^+), 513 (M + 2, 16), 515 (M + 4, 6); Anal. Calcd. For $C_{25}H_{24}N_6SCl_2$: C, 58.73; H, 4.69; N, 16.43%. Found: C, 58.85; H, 4.62; N, 16.38%

*6.12. 1-(4-(4′-chlorophenyl)-2-thiazolyl)-3-(4-methoxyphenyl)-5-(2-butyl-4-chloro-1H-imidazol-5yl)-2-pyrazoline (**IIIi**)*

IR (KBr, cm^{-1}): 1599 (–C═N), 3333 (–NH); ^1H NMR (DMSO-d_6, δ ppm): 0.91 (t, *J* = 7.2 Hz, 3H, $–CH_3$), 1.32 (m, 2H, $–CH_2–$), 1.65 (m, 2H, $–CH_2–$), 3.41 (s, 3H, OCH_3), 2.76 (t, *J* = 7.5 Hz, 2H, $–CH_2$), 3.48 (dd, 1H, *J* = 18.3, 8.1 Hz), 4.11 (dd, 1H, *J* = 18.3, 12.0 Hz), 5.62 (dd, 1H, *J* = 12.0, 8.1 Hz), 7.08 (s, 1H, -5H of thiazole), 7.21–8.15 (m, 8H, Ar–H), 8.19 (s, 1H, –NH, D_2O exchangeable); EIMS (*m*/*z*): 526 (M^+), 528 (M + 2, 38), 530 (M + 4, 8); Anal. Calcd. For $C_{26}H_{25}ON_5SCl_2$: C, 59.34; H, 4.75; N, 13.30%. Found: C, 59.21; H, 4.82; N, 13.16%

*6.13. 1-(4-(4′-chlorophenyl)-2-thiazolyl)-3-(2-hydroxy-3-iodo-5-methylphenyl)-5-(2-butyl-4-chloro-1H-imidazol-5yl)-2-pyrazoline (**IIIj**)*

IR (KBr, cm^{-1}): 1601 (–C═N), 3154 (–OH), 3331 (–NH); ^1H NMR (DMSO-d_6, δ ppm): 0.91 (t, *J* = 7.2 Hz, 3H, $–CH_3$), 1.31 (m, 2H, $–CH_2–$), 1.61 (m, 2H, $–CH_2–$), 2.41 (s, 3H, CH_3), 2.76 (t, *J* = 7.8 Hz, 2H, $–CH_2$), 3.54 (dd, 1H, *J* = 17.7, 8.4 Hz), 4.13 (dd, 1H, *J* = 17.7, 12.3 Hz), 5.61 (dd, 1H, *J* = 12.3, 8.4 Hz), 7.06 (s, 1H, -5H of thiazole), 7.23–8.18 (m, 6H, Ar–H), 8.21 (s, 1H, –NH, D_2O exchangeable), 12.48 (s, 1H, –OH, D_2O exchangeable); EIMS (*m*/*z*): 652 (M^+), 654 (M + 2, 26), 656 (M + 4, 5); Anal. Calcd. For $C_{26}H_{24}ON_5SCl_2I$: C, 47.87; H, 3.73; N, 10.72%. Found: C, 47.76; H, 3.78; N, 10.65%

*6.14. 1-(4-(4′-chlorophenyl)-2-thiazolyl)-3-(2-hydroxy-5-methylphenyl)-5-(2-butyl-4-chloro-1H-imidazol-5yl)-2-pyrazoline (**IIIl**)*

IR (KBr, cm^{-1}): 1599 (–C═N), 3136 (–OH), 3335 (–NH); ^1H NMR (DMSO-d_6, δ ppm): 0.91 (t, *J* = 7.5 Hz, 3H, $–CH_3$), 1.33 (m, 2H, $–CH_2–$), 1.64 (m, 2H, $–CH_2–$), 2.34 (s, 3H, CH_3), 2.75 (t, *J* = 7.8 Hz, 2H, $–CH_2$), 3.51 (dd, 1H, *J* = 18.0, 8.1 Hz), 4.09 (dd, 1H, *J* = 18.0, 12.0 Hz), 5.58 (dd, 1H, *J* = 12.0, 8.1 Hz), 7.05 (s, 1H, -5H of thiazole), 7.25–8.16 (m, 7H, Ar–H), 8.19 (s, 1H, –NH, D_2O exchangeable), 12.32 (s, 1H, –OH, D_2O exchangeable); EIMS (*m*/*z*): 526 (M^+), 528 (M + 2, 30), 530 (M + 4, 9); Anal. Calcd. For $C_{26}H_{25}ON_5SCl_2$: C, 59.34; H, 4.75; N, 13.30%. Found: C, 59.42; H, 4.78; N, 13.41%

*6.15. 1-(4-(4′-chlorophenyl)-2-thiazolyl)-3-(2-hydroxyphenyl)-5-(2-butyl-4-chloro-1H-imidazol-5yl)-2-pyrazoline (**IIIm**)*

IR (KBr, cm^{-1}): 1601 (–C═N), 3128 (–OH), 3335 (–NH); ^1H NMR (DMSO-d_6, δ ppm): 0.93 (t, *J* = 7.2 Hz, 3H, $–CH_3$), 1.32 (m, 2H, $–CH_2–$), 1.65 (m, 2H, $–CH_2–$), 2.76 (t, *J* = 7.5 Hz, 2H, $–CH_2$), 3.46 (dd, 1H, *J* = 18.3, 8.4 Hz – H_A), 4.08 (dd, 1H, *J* = 18.3, 12.3 Hz), 5.52 (dd, 1H, *J* = 12.3, 8.4 Hz), 7.05 (s, 1H, -5H of thiazole), 7.23–8.16 (m, 8H, Ar–H), 8.21 (s, 1H, –NH, D_2O exchangeable), 12.43 (s, 1H, –OH, D_2O exchangeable); EIMS (*m*/*z*): 512 (M^+), 514 (M + 2, 20), 516 (M + 4, 5); Anal. Calcd. For $C_{25}H_{23}ON_5SCl_2$: C, 58.62; H, 4.49; N, 13.66%. Found: C, 58.71; H, 4.58; N, 13.58%

*6.16. 1-(4-(4′-chlorophenyl)-2-thiazolyl)-3-(3,5-dibromo-2-hydroxyphenyl)-5-(2-butyl-4-chloro-1H-imidazol-5yl)-2-pyrazoline (**IIIo**)*

IR (KBr, cm^{-1}): 1605 (–C═N), 3142 (–OH), 3331 (–NH); ^1H NMR (DMSO-d_6, δ ppm): 0.91 (t, *J* = 7.2 Hz, 3H, $–CH_3$), 1.31 (m, 2H, $–CH_2–$), 1.62 (m, 2H, $–CH_2–$), 2.75 (t, *J* = 7.5 Hz, 2H, $–CH_2$), 3.52 (dd, 1H, *J* = 18.0, 8.1 Hz), 4.11 (dd, 1H, *J* = 18.0, 12.3 Hz), 5.56 (dd, 1H, J = 12.3, 8.1 Hz), 7.08 (s, 1H, -5H of thiazole), 7.23–8.18 (m, 6H, Ar–H), 8.20 (s, 1H, –NH, D_2O exchangeable), 12.38 (s, 1H, –OH, D_2O exchangeable); EIMS (*m*/*z*): 670 (M^+), 672 (M + 2, 32), 674 (M + 4, 18), 676 (M + 6, 4); Anal. Calcd. For $C_{25}H_{21}ON_5SCl_2Br_2$: C, 44.81; H, 3.13; N, 10.44%. Found: C, 44.72; H, 3.25; N, 10.36%

*6.17. 1-(4-(4′-chlorophenyl)-2-thiazolyl)-3-(3,5-dichloro-2-hydroxyphenyl)-5-(2-butyl-4-chloro-1H-imidazol-5yl)-2-pyrazoline (**IIIp**)*

IR (KBr, cm^{-1}): 1600 (–C═N), 3151 (–OH), 3338 (–NH); ^1H NMR (DMSO-d_6, δ ppm): 0.91 (t, *J* = 7.5 Hz, 3H, $–CH_3$), 1.32 (m, 2H, $–CH_2–$), 1.65 (m, 2H, $–CH_2–$), 2.76 (t, *J* = 7.5 Hz, 2H, $–CH_2$), 3.63 (dd, 1H, *J* = 17.7, 8.1 Hz), 4.16 (dd, 1H, *J* = 17.7, 12.3 Hz), 5.66 (dd, 1H, *J* = 12.3, 8.1 Hz), 7.10 (s, 1H, -5H of thiazole), 7.25–8.18 (m, 6H, Ar–H), 8.21 (s, 1H, –NH, D_2O exchangeable), 12.52 (s, 1H, –OH, D_2O exchangeable); EIMS (*m*/*z*): 581 (M^+), 583 (M + 2, 46), 585 (M + 4, 25), 587 (M + 6, 8), 589 (M + 8, 2); Anal. Calcd. For $C_{25}H_{21}ON_5SCl_4$: C, 51.66; H, 3.61; N, 12.04%. Found: C, 51.78; H, 3.68; N, 12.11%

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2009.10.015

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