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*Research Article*

# Design, Synthesis, and *In Vitro* Antioxidant Activity of 1,3,5-Trisubstituted-2-pyrazolines Derivatives

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Mannich base of pyrazolines 3(a–e) under both conventional and microwave irradiation was synthesized. All the synthesised compounds were purified by recrystallisation, characterized on the basis of UV, IR, and NMR spectroscopy, and further supported by mass spectroscopy. The result obtained confirms superiority of microwave irradiation method over classical heating one. The molecular properties and Lipinski rule of five for compounds 3(a–e) were determined by Molinspiration. The synthesized compounds were subsequently evaluated for the antioxidant activity. All the compounds were found in compliance with Lipinski "Rule of Five", and compound 3e having *p*-hydroxyl substitution showed best antioxidant activity as compared to ascorbic acid and rutin.

## 1. Introduction

Variously substituted pyrazolines and their derivatives are important biological agents, and a significant amount of research activity has been directed towards this class. In particular, they are used as antibacterial [1], antifungal [2], anti-inflammatory [3], central nervous system depressant [4], analgesic [5], mono amino oxidase inhibitor [6], anticancer [7], and antiviral agents [8]. Moreover, pyrazolines have played a crucial part in the development of theory in heterocyclic chemistry.

The advantages of microwave technology over conventional method have promoted us to synthesize pyrazolines from both conventional as well as microwave assisted method. There are several medical emergencies that result from free radical mediated pathology. The purpose of this study was to synthesize the pyrazoline derivatives in search for exploring the potential of this skeleton as antioxidant.

## 2. Materials and Methods

All the melting points reported were determined in open capillaries using Veego VMP-1 melting point apparatus expressed in °C and are uncorrected. The I. R. spectra of the compounds were recorded on Perkin-Elmer infrared 283 spectrometer in KBr disc and expressed in $cm^{-1}$. NMR spectra were recorded on Bruker DRX-300 spectrometer. Mass spectra were recorded on Jeol SX-102 (FAB) spectrometer. Synthesis and analytical studies of title compounds were carried out using laboratory grade reagents. Standard techniques like TLC were used to monitor reactions and to determine purity of the products. All the products were purified by recrystallization. The microwave-assisted syntheses were carried out in domestic oven, Midea PJ21B-A 400 W. All the compounds were subjected to molecular properties prediction and drug-likeness by Molinspiration software.

### 2.1. Procedure for Synthesis

*2.1.1. Step 1: Synthesis of Chalcones (3-Furan-2-yl-1-aryl-prop-2-en-1-one) 1(a–e).* To a solution of ketone (0.01 mole) and furfuraldehyde (0.01 mole) in 30 mL methanol, catalytic quantity of solid sodium hydroxide pellets were added and stirred at room temperature. The solution was neutralized with diluted HCl. The precipitated product was filtered,

washed with water, dried, and recrystallised from ethanol. The reaction was monitored by TLC using Toulene : Ethyl acetate (8 : 2) solvent system. By adopting this general procedure, five different chalcones were prepared using five different acetophenones [9].

*2.1.2. Step 2: Synthesis of 5-Furan-2-yl-3-aryl-4, 5-Dihydro-1H-pyrazole 2(a–e)*

*Conventional Method*. A mixture of chalcone 1(a–e) (0.01 mole) and hydrazine hydrate (0.02 mole) in 20 mL ethanol was refluxed for 4 to 6 hr; reaction was monitored by TLC and the resulting solution was left overnight in a refrigerator. Crystals separated were filtered and recrystalised from ethanol.

*Microwave Irradiation Method*. A mixture of chalcone 1(a–e) (0.01 mole) and hydrazine hydrate (0.02 mole) in 20 mL ethanol was subjected to microwave irradiation of 350 watt for a period of 2 to 3 minute. Reaction was monitored by TLC. Resulting solution was left overnight in refrigerator. Crystals separated were filtered and recrystalised from ethanol.

*2.1.3. Step 3: Synthesis of 5-Furan-2-yl-3-aryl-1-[(3-chlorophenyl) methyl amino]-2-pyrazoline 3(a–e).* A mixture of pyrazoline 2(a–e) (0.02 mole) and 3-chloro-aniline (0.02 mole), Paraformaldehyde (0.01 mole), and one drop of concentrated HCl in methanol (40 mL) was refluxed for 3-4 Hrs. Reaction was monitored by TLC. Excess solvent was removed by distillation under reduced pressure. After cooling the reaction mixture the product was separated out which was filtered and recrystallised from methanol.

*2.2. Molecular Properties Prediction and Drug-Likeness by Molinspiration*

*2.2.1. Molecular Properties Prediction.* Physicochemical parameters play a vital role in generation and determination of bioactivity of any compound. Molinspiration, web based software, was used to explore the various parameters such as miLogP, TPSA, MW, and drug likeness. MiLogP (octanol/water partition coefficient) was calculated by the method developed by Molinspiration as a sum of fragment based contributions and correction factors and used to predict the permeability of molecule across the cell membrane [10].

Topological Polar Surface Area (TPSA) is calculated based on the methodology published by Ertl et al. as the sum of fragment based contributions in which O- and N-centered polar fragments are to be considered and calculated by surface areas that are occupied by oxygen and nitrogen atoms and by hydrogen atoms attached to them [11]. TPSA has been used for characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability, and blood brain barrier permeability. Method for calculation of molecular volume developed by molinspiration is based on group contributions.

Number of rotatable bonds (nrotb) is a simple topological parameter that measures molecular flexibility. It has been shown to be a very good descriptor of oral bioavailability of drug [12]. Rotatable bond is defined as any single nonring bond, bounded to nonterminal heavy (i.e., nonhydrogen) atom. Amide C–N bonds are not considered because they are having high rotational energy barrier.

*2.2.2. Drug Likeness.* Drug likeliness is a qualitative means of analysis to check whether the given molecule has drug-like properties, and it is defined as a complex balance of various molecular properties and structural features. These properties, mainly hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size, and flexibility and presence of various pharmacophoric features, influence the behavior of a molecule in a living organism, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity, and metabolic stability [10].

*2.3. Antioxidant Activity*

*2.3.1. Scavenging of DPPH Radical [13].* Antioxidants react with stable free radical, DPPH, and convert it to 1,1-diphenyl-2-picryl hydrazine. The ability to scavenge the stable free radical DPPH was measured by decrease in absorbance at 517 nm. A standard drug ascorbic acid was also studied for comparison.

To the ethanolic solution of DPPH (0.5 mL of 0.0125 mM) an equal volume of the test compound dissolved in dimethyl sulfoxide [DMSO] was added at various concentrations; equal amount of DMSO was added to control solution. After 20 min at room temperature, absorbance at 517 nm was taken. The experiment was carried out in triplicate.

*2.3.2. Scavenging of Nitric Oxide [14].* Sodium nitroprusside spontaneously generates nitric oxide in aqueous solutions. Nitric oxide, generated in this manner, was converted to nitric and nitrous acids on contact with dissolved oxygen and water. The liberated nitrous acid is estimated using Greiss reagent. In presence of test components, likely to be scavengers, the amount of nitrous acid will decrease. The degree of decrease will reflect the extent of scavenging.

Sodium nitroprusside (5 mM) in PBS was incubated with different concentrations of test compound in DMSO at 25°C for 5 hrs. Control experiments without test compounds but with equivalent amounts of DMSO were conducted in an identical manner. After 5 hrs, 0.5 mL of incubated solution was removed and 0.5 mL of Greiss reagent was added. The absorbance of the chromophore formed during diazotisation of nitrite with sulfanilamide and subsequent coupling with N-(1-naphthyl) ethelene diamine was read at 546 nm. Experiment was carried out in triplicate.

## 3. Results and Discussion

*3.1. Chemistry.* In this present work, the reaction of chalcones 1(a–e) with various ketones under microwave assisted technique as well as conventional heating method was studied (Figure 1). The result obtained confirms the superiority of microwave irradiation method over classical heating one (Table 1).

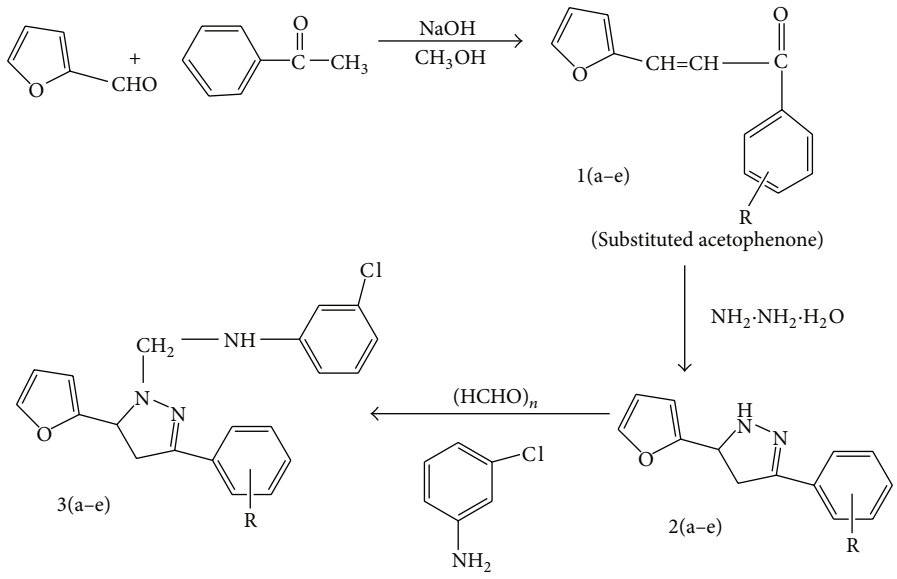



Figure 1: Scheme for synthesis of compounds 3(a–e).

Table 1: Comparative statement of synthesis under conventional and microwave technique.

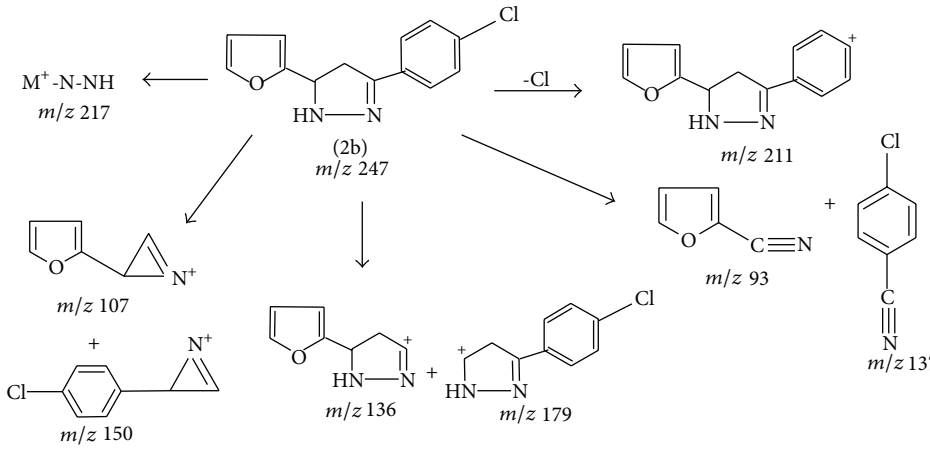
| S. no. | Product code | Conventional | | Microwave (350 watt) | |
|---|---|---|---|---|---|
| | | Reaction time (hours) | Yield % | Irradiation time (minute) | Yield % |
| (1) | 2a | 4-5 | 50 | 2.5 | 56 |
| (2) | 2b | 4-5 | 60 | 2.0 | 80 |
| (3) | 2c | 4-5 | 60 | 2.0 | 80 |
| (4) | 2d | 4-5 | 50 | 2.5 | 62 |
| (5) | 2e | 4-5 | 50 | 2.5 | 52 |

*3.1.1. Spectral Data and Interpretation of the Compounds* [2*b*], [2*c*], [2*e*], *and* [3*b*]. In the IR spectrum of compound 2e, it showed absorption band at 3318 $cm^{-1}$ corresponding to the pyrazoline NH group and OH group. The absorption bands at 1603 and 1515 $cm^{-1}$ are attributed to the C=N stretching and N–N stretching, respectively. In the IR spectrum of compound 2b showed absorption band at 3349.25 $cm^{-1}$ corresponding to the pyrazoline NH group. The absorption bands at 1592 and 1493.88 $cm^{-1}$ are attributed to the C=N stretching and N–N stretching, respectively. In the IR spectrum of compound 3b absorption bands appear at 3416, 1585, and 1496 $cm^{-1}$ for NH, C=N, and N–N groups, respectively. The absence of carbonyl band clearly supported the formation of 2-pyrazoline system.

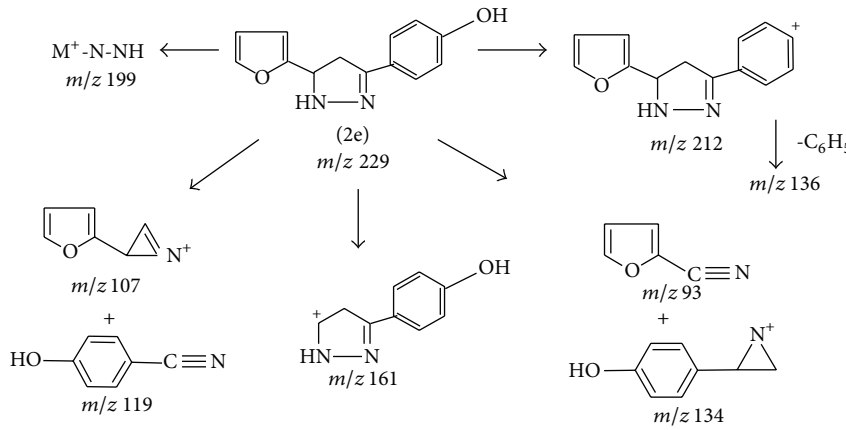
The $^1$H NMR spectrum of compounds 2c, 2b, 2e, and 3b exhibits a peak in the region $\delta$ 8.25 characteristic of NH proton. The doublet of doublets in the region $\delta$ 3.28 (1H, *d*, *d*, $J$ = 10, 10 Hz) and $\delta$ 3.38 (1H, *d*, *d*, $J$ = 11, 11 Hz) were due to the methylene protons of pyrazoline nucleus. The splitting is due to vicinal and geminal coupling of the methylene protons. Another doublet of doublet at $\delta$ 5.00 region (1H, *d*, *d*, $J$ = 10, 10 Hz) was due to the methine proton which was coupled with the magnetically nonequivalent methylene protons of pyrazoline ring. The doublets signals at $\delta$ 7.55 and 7.49 and the doublet of doublet at $\delta$ 6.30 region with a small coupling constant ($J$ = 4 Hz) are due to the furan ring system. The pair of doublet in the $\delta$ 7.5 region ($J$ = 8 Hz) are due to aromatic protons. The hydroxyl proton in compound 2e resonates at 4.95, and the aromatic proton signals in compound 3b resonate at $\delta$ 6.36 and 7.20 each integrating for two protons. All the signals in the compound 2e are in the upfield region when compared with the signals of compounds 2b, 2c, and 3b which may be due to the electron donating nature of the OH group.

In the compound 3b the presence of a singlet signal at $\delta$ 4.6 and the aromatic proton signals which resonate at $\delta$ 6.36 and 7.20 each integrating for two protons shows that the compound 2b has undergone Mannich reaction and yielded compound 3b.

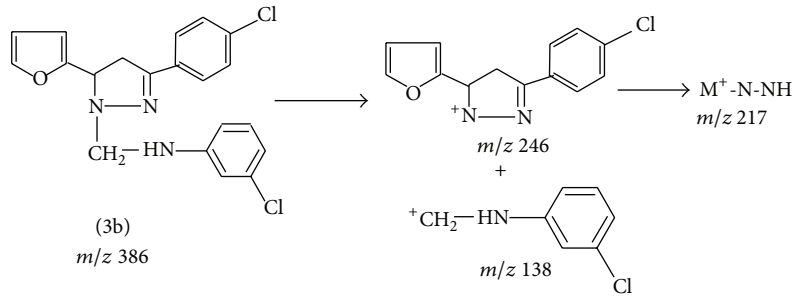
In the mass spectrum of compounds 2b and 2e, they exhibit molecular ion peaks at 246/248 and 228, which also includes the isotope peak of halogen atom. The compounds exhibit peaks at 217 and 199 due to $M^+$–N–NH characteristic of pyrazolines. The common peak at $m/z$ 93 and 107 present




Scheme 1: Fragmentation of Scheme 1.




Scheme 2: Fragmentation of Scheme 2.




Scheme 3: Fragmentation of Scheme 3.

Table 2: Molinspiration calculation for synthesized compounds (3a–3e).

| Compound | milogP | TPSA | natoms | MW | nON | nOHNH | Nviolation | Nrotb | Volume |
|---|---|---|---|---|---|---|---|---|---|
| 3a | 4.943 | 40.769 | 25 | 351.837 | 4 | 1 | 0 | 5 | 310.001 |
| 3b | 5.621 | 40.769 | 26 | 386.282 | 4 | 1 | 1 | 5 | 323.536 |
| 3c | 5.752 | 40.769 | 26 | 430.733 | 4 | 1 | 1 | 5 | 327.886 |
| 3d | 4.883 | 60.997 | 26 | 367.836 | 5 | 2 | 0 | 5 | 318.018 |
| 3e | 4.464 | 60.997 | 26 | 367.836 | 5 | 2 | 0 | 5 | 318.018 |
| Ascorbic acid | −1.402 | 107.217 | 12 | 176.124 | 6 | 4 | 0 | 2 | 139.707 |
| Rutin | −1.294 | 269.427 | 42 | 596.494 | 16 | 10 | 3 | 5 | 479.267 |

is due to furan nitrile and the furan nucleus, respectively. The p-chloro phenyl nitrile peak appears at $m/z$ 137 for compound 2b, and p-hydroxy phenyl nitrile peak appears at $m/z$ 119 for compound 2e as depicted in fragmentation Schemes 1 and 2.

In the mass spectrum of compound 3b $M^+$ ion peak is observed at 386/388, which includes isotope peak of halogen atom. The other $m/z$ peaks 246, 217, and 138 are due to fragments shown in fragmentation of Scheme 3.

*3.2. Molinspiration Calculation.* Results of compounds 3(a–e) in terms of molecular properties (molecular weight, milogP and TPSA, nrotb, volume, etc.) are valued in Table 2. According to Lipinski rule of five, most "drug-like" molecules have log $P \leq 5$, number of hydrogen bond acceptors ≤10, molecular weight ≤500, and number of hydrogen bond donors ≤5. Molecules violating more than one of these rules may have problems with bioavailability [15]. On close inspection of Table 2, all molecules were found in compliance with Lipinski rule of five recommendations for new chemical entity to have good oral bioavailability with no violations (Nviolation). The milog $P$ valve of all compounds was found below five, suggesting that the molecules have good permeability across the cell membrane which in turn is needed for generation of bioactivity. All the compounds (3a–3e) are within the limit, that is, 160 Å in respect of TPSA, which showed that molecules are fulfilling the optimal requirement for drug absorption.

*3.3. Antioxidant Activity*

*3.3.1. DPPH Radical Scavenging Activity.* The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. As shown in Tables 3 and 4, five compounds (2d, 2e, 3a, 3d, and 3e) showed radical scavenging activity. It is clear from the data that phenolic compounds are active (3e > 2e > 2d > 3d) and nonphenolic compounds are less or inactive. The scavenging activity order of the test compounds was 3e > 2e > 2d > 3a > 3d. The strong DPPH scavenging activity of can be attributed in part to the phenolic OH present in 3e, 2e, 2d, and 3d.

*3.3.2. Nitric Oxide Radical Scavenging Activity.* The effect of antioxidants on nitrogen centered nitric oxide radical scavenging. As shown in Tables 3 and 4, seven compounds (2d, 2e, 2a, 2b, 3a, 3d, and 3e) showed nitric oxide radical scavenging activity. The scavenging activity order of the test compounds was 3e > 3a > 2d > 2e > 3d > 2a > 2b.

## 4. Conclusion

As a result of our studies related to the development of synthetic protocols using both classical heating (conventional) method and microwave irradiation, we now report a novel and easy access to 1H-5(furan-2-yl) 3-aryl-2-pyrazolines using a one-pot procedure. This work demonstrates that all the synthesized compounds followed the Lipinski rule of five, that is, compounds may have the good oral bioavailability. By careful observation of the results of preliminary *in vitro* antioxidant activity of all the compounds, further screening on other *in vitro*, *ex vivo* and *in vivo* models will throw some light on the potential of compounds to be an antioxidant compound.

Table 3: Free radical scavenging activity of 5-furan-2-yl-3-aryl-4, 5-dihydro-1H-pyrazole 2(a–e).

| Compound | Concentration in $\mu$g/mL | Percentage of scavenging ± SD | |
|---|---|---|---|
| | | DPPH | Nitric oxide |
| 2a | 500 | 14.65 ± 0.324 | 61.06 ± 0.983 |
| | 250 | 06.32 ± 1.619 | 41.01 ± 1.280 |
| | 125 | 05.66 ± 0.081 | 31.05 ± 1.367 |
| 2b | 500 | 13.34 ± 0.371 | 42.51 ± 0.923 |
| | 250 | 07.81 ± 1.194 | 22.85 ± 0.976 |
| 2c | 500 | 14.46 ± 1.425 | 30.98 ± 0.596 |
| | 250 | 09.03 ± 0.493 | 26.23 ± 0.298 |
| 2d | 500 | 69.00 ± 0.986 | 67.25 ± 0.491 |
| | 250 | 68.39 ± 1.857 | 59.30 ± 0.406 |
| | 125 | 69.66 ± 0.854 | 55.79 ± 0.491 |
| | 62.5 | 46.20 ± 1.582 | 41.79 ± 0.390 |
| 2e | 500 | 74.34 ± 0.902 | 59.24 ± 0.112 |
| | 250 | 75.09 ± 1.054 | 49.93 ± 0.298 |
| | 125 | 64.84 ± 0.773 | 40.82 ± 0.516 |
| | 62.5 | 51.63 ± 1.475 | 33.00 ± 0.516 |
| | 31.25 | 21.91 ± 1.114 | — |
| Ascorbic acid | 50 | 50 ± 0.16 | — |
| Rutine | 50 | 70 ± 0.16 | 50 ± 0.11 |

Table 4: Free radical scavenging activity of 5-furan-2-yl-3-aryl-1-[(3-chloro-phenyl) methyl amino]-2-pyrazoline 3(a–e).

| Compound | Concentration in $\mu$g/mL | Percentage of scavenging ± SD | |
|---|---|---|---|
| | | DPPH | Nitric oxide |
| 3a | 500 | 57.16 ± 0.612 | 70.72 ± 0.331 |
| | 250 | 36.23 ± 0.506 | 63.60 ± 0.315 |
| | 125 | 18.82 ± 1.264 | 60.88 ± 0.103 |
| | 62.5 | 09.59 ± 0.858 | 56.38 ± 0.259 |
| 3b | 500 | 18.58 ± 1.615 | 32.37 ± 0.630 |
| | 250 | 07.16 ± 2.189 | 29.23 ± 0.622 |
| 3c | 500 | 04.63 ± 0.643 | 16.47 ± 0.930 |
| 3d | 500 | 48.50 ± 1.501 | 52.35 ± 0.157 |
| | 250 | 32.06 ± 1.778 | 46.43 ± 0.103 |
| | 125 | 19.56 ± 0.996 | 43.99 ± 0.214 |
| 3e | 500 | 85.06 ± 0.663 | 89.02 ± 0.487 |
| | 250 | 87.92 ± 0.612 | 91.12 ± 0.449 |
| | 125 | 85.95 ± 1.224 | 89.68 ± 0.899 |
| | 62.5 | 61.84 ± 1.733 | 71.96 ± 1.273 |
| | 31.25 | 37.26 ± 0.214 | 53.90 ± 0.157 |
| Ascorbic acid | 50 | 50 ± 0.16 | — |
| Rutine | 50 | 70 ± 0.16 | 50 ± 0.11 |

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## References

[1] E. M. Sharshira and N. M. M. Hamada, "Synthesis and antimicrobial evaluation of some pyrazole derivatives," *Molecules*, vol. 17, no. 5, pp. 4962–4971, 2012.

[2] H. Deng, Z. Y. Yu, G. Y. Shi, M. J. Chen, K. Tao, and T. Hou, "Synthesis and *in vitro* antifungal evaluation of 1,3,5-trisubstituted-2-pyrazoline derivatives," *Chemical Biology and Drug Design*, vol. 79, no. 3, pp. 279–289, 2012.

[3] M. Amir, H. Kumar, and S. A. Khan, "Synthesis and pharmacological evaluation of pyrazoline derivatives as new anti-inflammatory and analgesic agents," *Bioorganic and Medicinal Chemistry Letters*, vol. 18, no. 3, pp. 918–922, 2008.

[4] Z. Ozdemir, H. B. Kandilci, B. Gumusel, U. Calis, and A. A. Bilgin, "Synthesis and studies on antidepressant and anticonvulsant activities of some 3-(2-thienyl)pyrazoline derivatives," *Archiv der Pharmazie*, vol. 341, no. 11, pp. 701–707, 2008.

[5] S. Sridhar and Y. Rajendraprasad, "Synthesis and analgesic studies of some new 2-pyrazolines," *E-Journal of Chemistry*, vol. 9, no. 4, pp. 1810–1815, 2012.

[6] M. Karuppasamy, M. Mahapatra, S. Yabanoglu et al., "Development of selective and reversible pyrazoline based MAO-A inhibitors: synthesis, biological evaluation and docking studies," *Bioorganic and Medicinal Chemistry*, vol. 18, no. 5, pp. 1875–1881, 2010.

[7] R. Bashir, S. Ovais, S. Yaseen et al., "Synthesis of some new 1,3,5-trisubstituted pyrazolines bearing benzene sulfonamide as anticancer and anti-inflammatory agents," *Bioorganic and Medicinal Chemistry Letters*, vol. 21, no. 14, pp. 4301–4305, 2011.

[8] A. E. E. Amr, N. A. Abdel-Latif, and M. M. Abdalla, "Synthesis and antiandrogenic activity of some new 3-substituted androstano[17,16-c]-5′-aryl-pyrazoline and their derivatives," *Bioorganic and Medicinal Chemistry*, vol. 14, no. 2, pp. 373–384, 2006.

[9] S. Syam, S. I. Abdelwahab, M. A. Al-Mamary, and S. Mohan, "Synthesis of chalcones with anticancer activities," *Molecules*, vol. 17, no. 6, pp. 6179–6195, 2012.

[10] Molinspiration Cheminformatics [Homepage on the Internet], Nova Ulica, Slovensky Grob, Slovakia, 2012, http://www.molinspiration.com/.

[11] P. Ertl, B. Rohde, and P. Selzer, "Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties," *Journal of Medicinal Chemistry*, vol. 43, no. 20, pp. 3714–3717, 2000.

[12] D. F. Veber, S. R. Johnson, H. Cheng, B. R. Smith, K. W. Ward, and K. D. Kopple, "Molecular properties that influence the oral bioavailability of drug candidates," *Journal of Medicinal Chemistry*, vol. 45, no. 12, pp. 2615–2623, 2002.

[13] S. Sreejayan and M. N. A. Rao, "Oxygen free radical scavenging activity of the juice of *Momordica charantia* fruits," *Fitoterapia*, vol. 62, no. 4, pp. 344–346, 1991.

[14] L. Marcocci, J. J. Maguire, M. T. Droy-Lefaix, and L. Packer, "The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761," *Biochemical and Biophysical Research Communications*, vol. 201, no. 2, pp. 748–755, 1994.

[15] C. A. Lipinski, F. Lombardo, B. W. Dominy, and P. J. Feeney, "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings," *Advanced Drug Delivery Reviews*, vol. 23, no. 1–3, pp. 3–25, 1997.