Utility of Pyrazolylchalcone Synthon to Synthesize Azolopyrimidines under Grindstone Technology

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A series of pyrazolyl-triazolo[1,5-a]pyrimidines, pyrazolyl-tetrazolo[1,5-a]pyrimidines, pyrazolyl-benzo[4,5]imidazo[1,2-alpyrimidines and bis-azolopyrimidines were prepared by reaction of pyrazolyl-chalcones or its bis-pyrazolyl-chalcones with the appropriate heterocyclic amines as aminotriazole, aminotetrazole, 2-aminobenzimidazole and 4,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-3-amine by grinding method. The newly synthesized compounds have been characterized on the basis of elemental analysis and spectral data (IR, 1H- and 13C-NMR, Mass). Moreover, the newly synthesized products were screened for their in vitro antibacterial activities and the results showed that compounds 5f and 11d exhibited excellent activities compared with penicillin G and streptomycin as reference drugs.

Key words heterocyclic amine; pyrazolyl chalcone; azolopyrimidine; grinding; antibacterial activity

α,β-Unsaturated ketones display a wide range of pharmacological properties, including antimicrobial, antitumor, anxiolytic, antiviral, and antitubercular activities. 1–5 On the other hand, pyrazolines constitute an interesting class of heterocycles due to their synthetic versatility and effective biological activities such as antiviral, analgesic, anti-inflammatory, antidepressant, anticonvulsant, antibacterial, antifungal, antitubercular, antiinflammatory and anti-cancer activities. 6–14 Moreover, grinding method contributes to the development of a green strategy for the preparation of organic compounds in high yields with fewer waste products, simple, efficient, economical and environmentally benign compared to classical procedures. 15–20

In continuation of our previous work to discover new bioactive heterocyclic compounds, 21–31 we aimed here to synthesize new pyrazolyl-azolopyrimidines via ecofriendly method to evaluate their antibacterial activity.

Results and Discussion

Chemistry This article deals with the behavior of some heterocyclic amines toward α, β-unsaturated carbonyl compounds under ecofriendly conditions thus, when pyrazolyl chalcones 1a–f 25 were allowed to react with 5-amino-1,2,4-triazole via grinding in a mortar at room temperature, in the presence of few drops of glacial acetic acid as catalyst for 10–20 min, it yielded triazolopyrimidines 5a–f (Chart 1).

Similarly, when pyrazolyl chalcones 1a–c were submitted to react with 5-amino-tetrazole under the same conditions afforded the tetrazolopyrimidines 5g and h, respectively (Chart 1).

To account for the formation of products 5, it was suggested that intermediate 3 is initially formed via aza-Michael 1,4-addition of the amino group of compound 2 to the electron-deficient carbon of the chalcones 1 which undergoes dehydration cyclization to give the intermediate 4 which undergoes dehydrogenation leading to the more thermodynamically stable product 5 as shown in Chart 1.

Theoretically there are three paths for the reaction: Path (a) is a kinetically favor because the amino group at position 5 is sp2 hybridized and more nucleophilic than other nitrogen atoms (nucleophilicity is kinetically controlled), path (b) is not recommended because endocyclic amino group is sp2-hybridized and less nucleophilic and ruled out. Also path (c) is not acceptable because the carbonyl group of α,β-unsaturated compounds has weak electrophilic properties due to its conjugation with double bond, thus also can be ruled out. Thus compounds 8a–h are not obtained.

The elemental analysis together with the data derived from IR, 1H-NMR and mass spectra (MS) are in agreement with the proposed structure 5. The 1H-NMR spectra of compound 5a, taken as example, revealed the presence of three singlet signals at δ 2.60, 8.30 and 8.63 ppm, assigned for the aryl and pyrimidine protons. The MS of each of compounds 5 revealed the presence of a molecular ion peak (m/z) which is consistent with the structure of the respective compound (see Experimental).

Also when heterocyclic chalcones 1a, c, d and f were allowed to react with 2-aminobenzimidazole (10) under the same conditions, afforded 4-(5-methyl-1-phenyl-1H-pyrazol-4-yl)-2-aryl/or heteroaryl benzo[4,5]imidazo[1,2-alpyrimidine derivatives 11a–d, respectively, and not compounds 12a and b (Chart 2) as elucidated from spectroscopic tools (cf. Experimental).

In a similar manner, interaction of the heterocyclic chalcones 1a and c with 4,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-3-amine (13) under ecofriendly conditions, yielded pyrido[2′:3′:3,4] pyrazolo[1,5-a]pyrimidines 14a and b, respectively, and not compounds 15a and b (Chart 3). The structure 15 was substantiated by elemental analysis and spectral data. For example, the 1H-NMR spectra of products 15 revealed the presence of a characteristic singlet signal at δ 7.32 ppm, assigned for the pyridine proton, in addition to the expected signals of the methyl and aryl protons. The MS revealed a molecular ion peak at the correct values.
Chart 1. Synthesis of Compounds 5a–h

Chart 2. Synthesis of Compounds 11a–d
On the other hand, when the bis-chalcone 16 reacted with the appropriate 3-amino-1,2,4-triazole (2a), 5-aminotetrazole (2b), 2-aminobenzimidazole (10) and 4,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-3-amine (13), yielded the bis-aza Michael adduct 17a, b, 19 and 21 (Chart 4). The other products 18a, b, 20 and 22 were discarded because the nucleophilicity of the exo-amino group is higher than the endo-imino group. The structure of the products 7a–g was deduced from their spectral data (IR, 1H-NMR, and electrospray ionization (ESI)-MS) and elemental analyses, which are listed in Experimental.
Antimicrobial Evaluation The synthesized compounds were evaluated for their in vitro antibacterial activity at 5 mg/mL using agar well diffusion method against *Staphylococcus aureus* and *Bacillus subtilis* as examples of Gram-positive bacteria as well as *Pseudomonas aeruginosa* and *Escherichia coli* as examples of Gram-negative bacteria. The results of testing for antibacterial effects summarized in Table 1 showed that the new derivatives tested displayed variable in vitro antibacterial actions. In general, the chemical structure of the whole molecule, comprising the nature of the heterocyclic system as well as the type of the substituted function present in the heterocyclic ring structure, has a pronounced effect on activity (Fig. 1).

**From the screening results, it can be seen that:**

- Compounds 5h and 11d showed excellent activity against Gram-positive bacteria and Gram-negative bacteria comparable to the reference drugs.
- All the tested compounds have more activities towards Gram-positive bacteria than Gram-negative bacteria.
- For Gram-positive bacteria, most of the tested compounds have more activities towards *Bacillus subtilis* than *Staphylococcus aureus* as Gram-positive bacteria than Gram-negative bacteria.
- For Gram-negative bacteria, most of the tested compounds have more activities towards *Pseudomonas aeruginosa* than *Escherichia coli*.
- Bis-derivatives 17a, b and 19 showed no activities against bacteria.
- Most of the tested compounds have moderate activities towards bacteria.

**Experimental**

**Chemistry** All melting points (mp) were determined on an electrothermal Gallenkamp apparatus and are uncorrected. Solvents were generally distilled and dried by standard literature procedures prior to use. The IR spectra were measured on a Pye-Unicam SP300 instrument in potassium bromide discs. 1H-NMR (300 MHz), 13C-NMR (75 MHz) were run in deuterated dimethyl sulfoxide (DMSO-<sup>d6</sup>). Chemical shifts were related to that of the solvent. The MS were recorded on
General Method
A mixture of pyrazolyl-chalcone 1 (1 mmol) and the appropriate heterocyclic amine (2a, b or 10 or 13) (1 mmol) was grind with catalytic drops of acetic acid, in a mortar at room temperature for 10–20 min (monitored through TLC). The reaction mixture was poured into water and the solid product was collected by filtration followed by washing with ethanol. The crude product was then recrystallized from the appropriate solvent to give pure products 5a–h, 11a–d, and 14a, b respectively. Compounds 5a–h, 11a–d, and 14a, b with their physical constants and spectral data are depicted as shown below:

7-(5-Methyl-1-phenyl-1H-pyrazol-4-yl)-5-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine (5a)

Yellow solid (78%); mp 260–262°C (AcOH); IR: ν 1655 (C=O); 1H-NMR δ: 2.58 (s, 3H, CH3), 7.41–7.48 (s, 10H, Ar-H and pyrimidine-H), 8.30 (1H, s, triazole-H), 8.62, 9.32 (2s, 2H, 2pyrazole-H); MS m/z (%): 358 (M+, 22), 185 (31), 118 (26), 77 (100). Anal. Calcd for C20H15N7 (353.14): C, 67.98; H, 4.28; N, 27.75. Found: C, 67.85; H, 4.20; N, 27.66%.

5-(4-Chlorophenyl)-7-(5-methyl-1-phenyl-1H-pyrazol-4-yl)-4,7-dihydro[1,2,4]triazolo[1,5-a]pyrimidine (5g)

Yellow solid (78%); mp 260–262°C (AcOH); IR: ν 1651 (C=O); 1H-NMR δ: 2.59 (s, 3H, CH3), 7.51–7.92 (m, 10H, Ar-H and pyrimidine-H), 8.82 (s, 1H, pyrazole-H); MS m/z (%): 389 (M+, 4), 387 (M++, 15), 322 (31), 185 (44), 118 (21), 77 (100). Anal. Calcd for C19H14N6Cl (342.12): C, 66.66; H, 4.12; N, 25.21. Found: C, 67.85; H, 4.69; N, 17.35%.

4-(5-Methyl-1-phenyl-1H-pyrazol-4-yl)-2-phenylbenzo[4,5]imidazo[1,2-a]pyrimidine (11a)

Yellow solid (75%); mp 247–249°C (DMF); IR: ν 1652 (C=O); 1H-NMR δ: 2.59 (s, 3H, CH3), 7.51–7.92 (m, 15H, Ar-H and pyrimidine-H), 8.62 (s, 1H, pyrazole-H); MS m/z (%): 401 (M++ 25), 371 (65), 185 (89), 133 (25), 77 (100). Anal. Calcd for C22H17N5 (407.12): C, 71.64; H, 4.16; N, 16.07. Found: C, 71.51; H, 4.08; N, 16.02%.

4-(5-Methyl-1-phenyl-1H-pyrazol-4-yl)-2-(thiophen-2-yl)benzo[4,5]imidazo[1,2-a]pyrimidine (11e)

Yellow solid (73%); mp 241–243°C (AcOH); IR: ν 1655 (C=O); 1H-NMR δ: 2.58 (s, 3H, CH3), 7.17–7.80 (m, 13H, Ar-H and pyrimidine-H), 8.53 (s, 1H, pyrazole-H); MS m/z (%): 407 (M+, 14), 294 (31), 185 (100), 118 (31), 77 (81), 58 (92). Anal. Calcd for C25H19N5S (479.40): C, 70.74; H, 4.21; N, 17.19. Found: C, 70.72; H, 4.09; N, 17.10%. 

5-(1,3-Diphenyl-1H-pyrazol-4-yl)-4-(5-methyl-1-phenyl-1H-pyrazol-4-yl)benzo[4,5]imidazo[1,2-a]pyrimidine (11d)
Yellow solid (79%); mp 283–285°C (dioxane); IR: ν 1652 (C=N), 2924, 3059 (C–H) cm⁻¹; ¹H-NMR δ: 2.58 (s, 3H, CH₃), 7.41–7.96 (m, 20H, Ar-H and pyrimidine-H), 8.42 (s, 1H, pyrazole-H), 9.33 (s, 1H, pyrazole-H); MS m/z (%): 543 (M⁺, 19), 430 (24), 245 (50), 185 (72), 77 (100). Anal. Calcd for C₃₅H₂₅N₇ (543.22): C, 77.33; H, 4.64; N, 18.04. Found: C, 64.96; H, 3.85; N, 31.19. Found: C, 64.85; H, 3.69; N, 26.70%.

8,10-Dimethyl-4-(5-methyl-1-phenyl-1H-pyrazol-4-yl)-2-pyrenylidene(2′,3′,3′,4′)pyrazolo[1,5-alpyrimidine (14a)

Yellow solid (82%); mp 260–262°C (DMF); IR: ν 1650 (C=N), 2935, 2998, 3061 (C–H) cm⁻¹; ¹H-NMR δ: 2.59 (s, 3H, CH₃), 2.67 (s, 3H, CH₃), 2.85 (s, 3H, CH₃), 7.32 (s, 1H, pyridine-H), 7.43–7.92 (m, 11H, Ar-H and pyrimidine-H), 8.62 (s, 1H, pyrazole-H); MS m/z (%): 430 (M⁺, 13), 288 (62), 185 (100), 118 (26), 77 (61). Anal. Calcd for C₂₇H₂₁ClN₆ (464.15): C, 69.75; H, 4.55; N, 18.08. Found: C, 75.33; H, 5.15; N, 19.52. Found: C, 75.19; H, 5.11; N, 19.48%.

1,4-Bis[1,4-bis(7-(5-methyl-1-phenyl-1H-pyrazol-4-yl)tetrazolo)[1,5-a]pyrimidin-2-yl]benzene (21)

Yellow solid (74%); mp 294–296°C (DMF); IR: ν 1642 (C=N), 2921, 3056 (C–H) cm⁻¹; ¹H-NMR δ: 2.60 (s, 6H, 2CH₂), 2.82 (s, 6H, 2CH₂), 2.88 (s, 6H, 2CH₂), 7.15 (s, 2H, 2 pyridine-H), 7.50–7.60 (m, 10H, 1H, Ar-H), 7.81 (s, 2H, 2 pyrimidine-H 8.19 (s, 4H, Ar-H), 8.69 (s, 2H, 2 pyrazole-H); MS m/z (%): 782 (M⁺, 6), 444 (25), 317 (52), 185 (100), 77 (78), 58 (52). Anal. Calcd for C₄₆H₃₅N₁₂ (724.28): C, 73.64; H, 4.89; N, 21.47. Found: C, 73.42; H, 4.83; N, 21.33%

Antibacterial Activity Assay

The preliminary antimicrobial activity was investigated on a dozen of newly synthesized compounds in order to increase the selectivity of these derivatives towards test microorganisms. The antimicrobial profile was tested using a modified well diffusion method. Briefly, 100 μL of the test bacteria was grown in 10 mL of fresh media Mueller–Hinton and Sabouraud agar (Oxoid, U.K.) until they reached a count of approximately 10⁶ cells/mL. A hundred microliter of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained and tested for susceptibility by well diffusion method. A hundred microliter of each sample (at 5 mg/mL) was added to each well (10 mm diameter holes cut in the agar gel). The plates were incubated for 24–48 h at 37°C. After incubation, the microorganism’s growth was observed. The plates were done in triplicate and the resulting inhibition zone diameters were measured in millimeters and used as criterion for the antimicrobial activity. The size of the clear zone is proportional to the inhibitory action of the compound under investigation. Penicillin G and streptomycin (Sigma-Aldrich, U.S.A.) were used as a positive control against Gram-positive and Gram-negative bacteria, respectively. Solvent control (DMSO) was included in every experiment as negative control.

Conclusion

In conclusion, we have reported a simple and efficient solvent-free grinding method for synthesis of new azolopyrimidine derivatives and its bi-derivatives in good yields. The synthesized compounds were evaluated for their in vitro antibacterial activity at 5 mg/mL using agar well diffusion method against a representative panel of pathogenic strains and the results indicated that compounds 5f and 11d showed excellent activity against bacteria.

Conflict of Interest

The authors declare no conflict of interest.

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