SYNTHESIS AND ANTIMICROBIAL EVALUATION OF SOME NEW DIHYDROPYRIDINE, PYRAZOLE, CHROMENE, PYRROLE, THIAZOLE AND THIOPHENE DERIVATIVES

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Abstract – Synthesis of 2-cyano-N-((2-methoxynaphthalen-1-yl)methylene)-acetohydrazide (3) and its use as a key intermediate for the synthesis of some new heterocyclic compounds such as dihydropyridines (4, 6 and 8), pyrazoles (9 and 10), chromene (11), pyrroles (12 and 13), thiazoles (14 and 17) and thiophene (18-20) derivatives were described. The structures of newly synthesized compounds have been established on the basis of their IR, $^1$H-NMR, $^{13}$C-NMR and mass spectral data and have been screened for their antimicrobial activity.

INTRODUCTION

The hydrazide-hydrazone derivatives are important classes of heterocyclic compounds$^{1-3}$ possessing biological$^{4}$ and pharmacological activities such as antibacterial-antifungal,$^{5-7}$ anticonvulsant,$^{8,9}$ anti-inflammatory,$^{10,11}$ antimalarial$^{12}$ and antituberculosis activities.$^{13-15}$ In continuation of our interest in the synthesis of new heterocyclic compounds containing pyridine,$^{16}$ pyrazole,$^{17}$ chromene, pyrrole,
thiazole and thiophene moieties, we are interested in identifying new candidates that may be valuable in designing new, potent, selective and less toxic antimicrobial agents. We report herein the synthesis of hydrazide together with a series of heterocyclic transformation and their evaluation as antimicrobial agents. This combination was designed to investigate the influence of such hybridization and structure variation on the anticipated biological activities, hoping to add some synergistic biological significance to the target molecules.

RESULTS AND DISCUSSION

The synthetic procedures adopted to obtain the target compounds are depicted in Schemes 1-3. In Scheme 1, 1,6-diamino-4-(2-methoxynaphthalen-1-yl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (4) was synthesized in good yield by the condensation of 2-methoxy-1-naphthaldehyde (1) with malononitrile in refluxing ethanol catalyzed by TEA to give the corresponding derivative 2, followed by addition of 2-cyanoacetic acid hydrazide in ethanol and in the presence of a catalytic amount of piperidine. The assignment of structure 2 was supported by elemental analysis and spectral data. The IR spectrum showed the absorption band at 2226 cm\(^{-1}\) assignable to CN group. Its \(^1\)H-NMR spectrum exhibited a signal at \(\delta\) 8.95 ppm assignable to vinylic proton. On the other hand, the reaction of compound 1 with 2-cyanoacetic acid hydrazide in refluxing ethanol afforded 2-cyano-\(\text{n}=(\text{2-methoxynaphthalen-1-yl})\)-methylenacetohydrazide (3) which could be transformed to dihydropyridine derivative 4 upon heating with malononitrile in ethanol and in the presence of a catalytic amount of piperidine. The structures of compounds 3 and 4 were confirmed on the basis of the analytical and spectral data. The IR spectrum of compound 3 showed the absorption bands at 3190 and 2263 cm\(^{-1}\) due to NH and CN groups, respectively, in addition to stretching vibration of carbonyl group at 1685 cm\(^{-1}\). Its \(^1\)H-NMR spectrum revealed the presence of singlet signals at \(\delta\) 3.97, 4.24, 8.71 and 11.77 ppm assignable to O Me, CH\(_2\)CN, CH=N and NH protons, respectively. The mass spectrum showed the molecular ion peak at \(m/z\) 267 (m\(^+\), 77.06) corresponding to the molecular formula (C\(_{15}\)H\(_{13}\)N\(_3\)O\(_2\)). The mass spectrum of 4 showed the molecular ion peak at \(m/z\) 331 (m\(^+\), 57.60) which is in agreement with the expected molecular formula C\(_{18}\)H\(_{13}\)N\(_5\)O\(_2\). Equimolecular amount of compound 3 and chalcone 5 was refluxed in DMF in the presence of a catalytic amount of triethylamine, to yield the corresponding 1,2-dihydropyridine derivative 6. The structure of compound 6 was confirmed by elemental analysis and spectral data. The IR spectrum showed the absence of CN absorption band. Its \(^1\)H-NMR spectrum revealed singlet signal at \(\delta\) 6.12 ppm due to C\(_5\)-H of pyridine ring.
In a similar manner, treatment of compound 3 with enaminone 7 in refluxing glacial acetic acid gave the 1,2-dihydropyridine derivative 8. The structure of compound 8 was confirmed by elemental analysis and spectral data. The IR spectrum displayed stretching bands at 3428 and 1662 cm$^{-1}$ due to NH and CO groups, respectively. Its $^1$H-NMR spectrum showed two doublets at $\delta$ 8.69 and 6.13 ppm due to C$_4$-H and C$_5$-H of pyridine ring. The formation of compound 6 was assumed to proceed via Michael addition of the active methylene nitrile of 3 to $\alpha$,$\beta$-unsaturated ketone to yield the corresponding Michael adduct, followed by an intramolecular cyclization and Dimroth rearrangement following by autoxidation. On the other hand, compound 8 was formed according to the proposed mechanism as show in Scheme 1. Refluxing of compound 3 in DMF and triethylamine afforded the pyrazolone derivative 9. The assignment of structure 9 was supported by elemental analysis and spectral data. The IR spectrum showed the absorption bands at 3190, 2217 and 1671 cm$^{-1}$ assignable to NH, CN and carbonyl groups, respectively. We studied the reactivity of hydrazide-hydrazone derivative 3 towards the active methylene compounds. Thus, the reaction of compound 3 with ethyl cyanoacetate in DMF and a catalytic amount of TEA afforded the pyrazole derivative 10. The assignment of structure 10 was supported by elemental analysis and spectral data. The IR spectrum showed the absorption bands at 3333, 3190, 2264 and 1686 cm$^{-1}$ assignable to OH, NH, CN and carbonyl groups, respectively. Its $^1$H-NMR spectrum revealed two singlet signals at $\delta$ 4.23 and 5.04 ppm due to CH$_2$ and C$_5$-H of pyrazole ring. On the other hand, treatment of compound 3 with 2-hydroxy-1-naphthaldehyde in a basic medium afforded the chromene derivative 11. The assignment of structure 11 was supported by elemental analysis and spectral data. The IR spectrum revealed the absence of CN absorption band and the presence of new absorption band at 1713 cm$^{-1}$ assignable to lactone carbonyl group. Its $^1$H-NMR spectrum showed singlet signal at $\delta$ 8.46 ppm due to C$_4$-H of coumarin ring. Moreover, the reaction of hydrazide-hydrazone moiety with $\alpha$-halo compounds afforded the pyrrole derivatives. Therefore, treatment of compound 3 with either phenacyl bromide or chloroacetonitrile in the presence of DMF and triethylamine as a basic catalyst furnished the pyrrole derivatives 12 and 13, respectively. The structures 12 and 13, were assigned by elemental analysis and spectroscopic measurements. The IR spectra, in general, displayed stretching bands at 2212 and 1657 cm$^{-1}$ due to CN and C=O groups, respectively. The thiazoline heterocyclic ring incorporating a hydrazide-hydrazone moiety was prepared from compound 3. Thus, treatment of 3 with a mixture of sulfur and phenyl isothiocyanate in DMF and excess of TEA gave n-phenylthiazole derivative 14 that is in complete agreement with mass spectral measurements and analytical data (Scheme 3).
The structure 14 could be established for the reaction product based on the IR spectrum which showed absorption bands at 3423 and 3345 cm$^{-1}$ (NH$_2$). Its $^1$H-NMR spectrum revealed the presence of signals at $\delta$ 6.69 ppm due to NH$_2$ protons. The reactivity of 3 with isothiocyanates was examined. Thus, treatment of an equimolar amount of compound 3, phenyl isothiocyanate and potassium hydroxide in the presence of dimethylformamide yielded the non-isolable intermediate potassium sulfide salt 15, which by addition of dilute hydrochloric acid furnished the corresponding thiocarbamoyl derivative 16. The assignment of structure 16 was supported by elemental analysis and spectral data. The IR spectrum revealed the absorption bands at 3187, 2262, 1685 and 1270 cm$^{-1}$ assignable to NH, CN, CO and C=S groups, respectively. When compound 16 was treated with an equimolar amount of chloroacetyl chloride in DMF and TEA, 2-cyano-n-((2-methoxynaphthalen-1-yl)methylene)-2-(5-oxo-3-phenylthiazolidin-2-ylidene)acetohydrazi-de (17) was obtained. The assignment of structure 17 was supported by elemental analysis and spectral data. The IR spectrum revealed the appearance of cyano absorption band at 2263 cm$^{-1}$. Its $^1$H-NMR spectrum (DMSO-$d_6$) revealed the presence of a singlet signal at $\delta$ 4.22 ppm assignable to C$_5$-H thiazoline proton. Finally, the thiocarbamoyl derivative 16 reacted with each of
chloroacetone, phenacyl bromide and ethyl chloroacetate in refluxing DMF containing a catalytic amount of triethylamine to afford a single product, which in each case, was identified as the aminothiophene derivatives 18-20, respectively, based on the elemental analysis and spectral data of isolated products (see experimental).

\[ \text{Scheme 3} \]

**BIOLOGICAL ACTIVITY**

**ANTIMICROBIAL EVALUATION**

All the newly synthesized compounds 3-20 were evaluated for *in vitro* antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*) (MTCC-96) and Gram-negative bacteria (*Escherichia coli*) (MTCC-443) and fungal (*Candida Albicans*) using conventional Broth dilution method. The individual minimum inhibitory concentration (MIC, µg/mL) values of tested compounds against the
microbes are listed in Table 1 along with MIC values of reference compounds Ampicillin (for bacteria) and Clotrimazole (for fungi). From antibacterial activity data (Table 1), it was observed that compounds 2 and 3 exhibited the highest activity against \textit{S. aureus}. Compounds 12 and 17-20 exhibited high activity against \textit{E. coli}. When formyl group in compound 1 was replaced by cyanomethyleneacetohydrazide and malononitrile moieties, (compounds 2 and 3), the activity increased and displayed the highest activity at (MIC = 62.5 \(\mu\)g/mL) against \textit{S. aureus}, while compounds 13 and 20 which contain pyrrole and thiophene moieties showed good activity (MIC = 125 \(\mu\)g/mL) against \textit{S. aureus} bacteria. On the other hand, compounds 10 and 17 were equipotent to Ampicillin in inhibiting the growth of \textit{S. aureus} (MIC = 187.5 \(\mu\)g/mL). On the other hand, compounds 2, 3 and 10 exhibited equipotent to Ampicillin in inhibiting the growth of \textit{E. coli} (MIC = 125 \(\mu\)g/mL). Among these compounds, 12, 17 and 18 showed the highest activity profile against \textit{E. coli} (MIC = 3.9 \(\mu\)g/mL), while compounds 19 and 20 revealed very good growth in inhibitory activity. Compounds 2-20 were also evaluated for their \textit{in vitro} antifungal activity against \textit{C. albicans}. All compounds exhibited a very weak activity against \textit{C. albicans} if they are compared to standard Clotrimazole (MIC = 125-500 \(\mu\)g/mL).

**STRUCTURE ACTIVITY RELATIONSHIPS (SAR'S):**

From the results of antimicrobial activity of the newly synthesized compounds 3-20, antimicrobial activity was considerably affected by electron withdrawing substituents. The incorporation of electron withdrawing groups is responsible for enhancing activity against the test microorganism. The role of electron withdrawing group in improving antimicrobial activity is very well supported by previous studies.\textsuperscript{21,22} In case of antibacterial activity of compound 3 which contains cyanomethyleneacetohydrazide moiety with electron withdrawing properties is most potent against \textit{S. aureus}, while compounds 12 and 17-20 which contain heterocyclic ring pyrrole, thiazole and thiophene moieties with electron withdrawing properties were most potent against \textit{E. coli}. Moreover, the newly synthesized compounds have higher potency against Gram-negative bacteria than Gram-positive bacterial strains. In addition, antibacterial activity showed that thiophene ring displayed higher activity than the other heterocyclic rings. It may be attributed to the high electronegativity and consequently the electron withdrawing properties of sulfur atom. On the other hand, antifungal activity displayed conflicting results wherein compounds having electron withdrawing substituents revealed weak inhibition.
Table 1. Minimal inhibitory concentration (mic, µg/mL) of the newly synthesized compounds

<table>
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<tr>
<th>Compound No.</th>
<th>MIC, in µg/mL</th>
<th>Gram(+)bacteria</th>
<th>Gram(-)bacteria</th>
<th>fungi</th>
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<tr>
<td></td>
<td>S. aureus</td>
<td>E. coli</td>
<td>C. albicans</td>
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<tr>
<td>2</td>
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<td>125</td>
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<tr>
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<td>250</td>
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<td>NA</td>
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"NA": No activity

In conclusion, our attempts at exploring hydrazide-hydrazone based on pyridine, pyrazoline, pyrane, pyrrole, thiazoline and thiophene derivatives have unexpectedly led to identification of a novel chemo type with substantial antimicrobial activity. Among the newly synthesized compounds, 3-20 analogs 3, 12 and 17-20 showed highest inhibition against nearly all of the tested bacteria, while all compounds were inactive against fungal strains. Results of antimicrobial activity clearly demonstrated that the presence of electron withdrawing groups/atoms attached to the hydrazide-hydrazone is essential for enhancing antimicrobial activity. On the basis of structure activity relationship, electron withdrawing substituents
(thiophene ring) are beneficial for antibacterial activity and inactive for antifungal activity. From the activity data, compounds 2 and 3 showed highest antibacterial inhibition against *S. aureus*, while compounds 12, 17 and 18 showed highest antibacterial inhibition against *E. coli*. Thus, suggesting that the compounds from the present series with electron withdrawing groups can serve as important gateways for the design and development of new antimicrobial agents with potent activity and minimal toxicity.

**EXPERIMENTAL**

Melting points measured with a Gallenkamp apparatus are uncorrected. IR spectra were recorded KBr disc on a Mattson 5000 FTIR spectrophotometer at Microanalytical Unit, Faculty of Science, Mansoura University. The $^1$H NMR and $^{13}$C NMR spectra were measured on Bruker WP AC 300 (300 and 75 MHz) in DMSO-$d_6$ as a solvent, using tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed as $\delta_{ppm}$. Mass spectra were determined on Finnigan Incos 500 (70 eV). Elemental analyses were carried out at the Microanalytical Centre, Faculty of Science, Cairo University.

**Synthesis of 2-((2-methoxynaphthalen-1-yl)methylene)malononitrile (2).** To a solution of compound 1 (0.93 g, 5 mmol) in EtOH (20 mL), triethylamine (0.5 mL) and malononitrile (0.33 g, 5 mmol) were added. The reaction mixture was heated under reflux for 2 h, and then allowed to cool. The precipitate was filtered off and recrystallized from EtOH to afford 2; yellow crystals; yield (89.8%); mp 160-162 °C; IR (KBr): $\nu/$cm$^{-1}$ = 2226 (CN), $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$ (ppm): 4.05 (s, 3H, OCH$_3$), 8.95 (s, 1H, =CH), 7.46-8.24 (m, 6, Ar-H). MS (EI, 70 eV) m/z (%) = 234 (M$^+$, 100.00), 219 (22.42), 203 (13.86), 191 (38.74), 164 (73.36), 141 (15.69). Anal. Calcd for C$_{15}$H$_{10}$N$_2$O (234.26): C 76.91; H 4.30; N 11.96%. Found: C 76.90; H 4.33; N 11.94%.

**Synthesis of 2-cyano-N-((2-methoxynaphthalen-1-yl)methylene)acetohydrazide (3).** A mixture of compound 1 (3.7 g, 20 mmol) and 2-cyanoacetohydrazide (1.98 g, 20 mmol) in absolute EtOH (20 mL) was refluxed for 2 h, and the obtained solid was filtered off while hot and recrystallized from EtOH to give the title compound 3; white crystals; yield (95.3%); mp 220-222 °C; IR (KBr): $\nu/$cm$^{-1}$ = 3190 (NH), 2263 (CN), 1685 (CO); $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$ (ppm): 3.97 (s, 3H, OCH$_3$), 4.24 (s, 2H, CH$_2$), 7.39-8.08 (m, 6H, Ar-H), 8.71 (s,1H, CH=N), 11.77 (s, 1H, NH); $^{13}$C-NMR (75 MHz, DMSO-$d_6$) $\delta$ (ppm): 29.4, 56.1, 106.0, 117.2, 118.2, 123.4, 124.5, 126.3, 128.3 (2C), 129.2, 133.6, 142.7, 153.2, 172.0; MS (EI, 70 eV) m/z (%) = 267 (M$^+$, 77.06), 183 (100.00) 140 (19.20), 128 (17.22). Anal. Calcd for C$_{15}$H$_{13}$N$_3$O$_2$ (267.29): C 67.40; H 4.90; N 15.72%. Found: C 67.42; H 4.89; N 15.70%.

**Synthesis of 1,6-diamino-4-(2-methoxynaphthalen-1-yl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (4). Method A:** A mixture of compound 2 (2.34 g, 10 mmol) and cyanoacetohydrazide (0.99 g, 10 mmol) in absolute EtOH (50 mL) containing two drops of piperidine, was heated under reflux
for 3 h. The orange yellow precipitate obtained during heating was filtered and crystallized from EtOH to give compound 4 as orange-yellow crystals; yield (75%); mp 284-286 °C.

**Method B:** A mixture of compound 3 (1.33 g, 5 mmol) and malononitrile (0.33 g, 5 mmol) in absolute EtOH (30 mL) containing two drops of piperidine, was heated under reflux for 3 h. The orange yellow precipitate obtained during heating was filtered and crystallized from EtOH to give compound 4; orange-yellow crystals; yield (68%); mp 284-286 °C; IR (KBr): \( \nu / \text{cm}^{-1} = 3424, 3393 (2\text{NH}_2), 2249, 2176 (2\text{CN}), 1682 (\text{CO}) \); \(^1\text{H}-\text{NMR} (300 \text{ MHz, DMSO-d}_6) \ \delta (\text{ppm}): 3.91 (s, 3\text{H}, \text{OCH}_3), 4.79 (bs, 2\text{H}, \text{N-NH}_2), 5.47 (bs, 2\text{H}, \text{C-NH}_2), 7.37-8.51 (m, 6\text{H}, \text{Ar-H}); \(^{13}\text{C}-\text{NMR} (75 \text{ MHz, DMSO-d}_6) \ \delta (\text{ppm}): 56.2, 76.4, 101.4, 115.5 (3\text{C}), 118.2, 123.4, 124.5, 126.8, 128.5, 129.1 (2\text{C}), 132.0, 153.1, 159.5, 161.5, 169.8; MS (EI, 70 eV) \( m/\text{z} \) (%) = 331 (M\(^+\), 57.60), 306 (81.50), 289 (32.50), 275 (23.20), 235 (63.60), 158 (100.00), 139 (30.50).

Anal. Calcd for C\(_{18}\)H\(_{13}\)N\(_5\)O\(_2\) (331.33): C 65.25; H 3.95; N 21.14%. Found: C 65.26; H 3.92; N 21.08%

**Synthesis of N-((2-methoxynaphthalen-1-yl)methylene)-2-oxo-4,6-diphenyl-1,2-dihydropyridine-3-carbohydrazide (6).** To a solution of compound 3 (0.53 g, 2 mmol) in DMF (10 mL) containing triethylamine (0.5 mL), chalcone (5) (0.41 g, 2 mmol) was added. The reaction mixture was heated under reflux for 5 h, and then allowed to cool. The precipitate that formed was filtered off and recrystallized from EtOH to afford 6; yellow powder; yield (70.5%); mp 265-267 °C; IR (KBr): \( \nu / \text{cm}^{-1} = 3443 (\text{NH}), 1682, 1628 (2\text{CO}) \); \(^1\text{H}-\text{NMR} (300 \text{ MHz, DMSO-d}_6) \ \delta (\text{ppm}): 4.06 (s, 3\text{H}, \text{OCH}_3), 6.12 (s, 1\text{H}, \text{C5-H of pyridine}), 7.44-8.16 (m, 16\text{H}, \text{Ar-H}), 9.02 (s, 1\text{H}, \text{CH=N}), 9.49 (s, 1\text{H}, \text{NH}), 11.75, (s, 1\text{H}, \text{NHCO}); \(^{13}\text{C}-\text{NMR} (75 \text{ MHz, DMSO-d}_6) \ \delta (\text{ppm}): 56.1, 104.8, 106.5, 118.2, 120.2, 123.4, 124.5, 126.8, 127.9 (2\text{C}), 128.2 (4\text{C}), 128.6 (4\text{C}), 129.1(3\text{C}), 130.6, 133.6, 140.8 (2\text{C}), 142.7, 153.3, 160.1, 161.5, 172.0; MS (EI, 70 eV) \( m/\text{z} \) (%) = 473 (M\(^+\), 66.70), 283 (66.70), 183 (44.40), 140 (77.80). Anal. Calcd for C\(_{30}\)H\(_{23}\)N\(_3\)O\(_3\) (473.53): C 76.09; H 4.90; N 8.87%. Found: C 76.05; H 4.88; N 8.84%.

**Synthesis of N-((2-methoxynaphthalen-1-yl)methylene)-2-oxo-6-phenyl-1,2-dihydropyridine-3-carbohydrazide (8).** A mixture of compound 3 (2.34 g, 10 mmol) and enaminone 7 (1.75 g, 10 mmol) was refluxed in glacial acetic acid (20 mL) in presence of anhydrous sodium acetate (2 g) for 4 h. Then, the reaction mixture was allowed to cool. The precipitate that formed was filtered off and recrystallized from EtOH to afford 8; orange crystals; yield (73%); mp 197-198 °C; IR (KBr): \( \nu / \text{cm}^{-1} = 3428 (2\text{NH}), 1662-1624 (2\text{CO}) \); \(^1\text{H}-\text{NMR} (300 \text{ MHz, DMSO-d}_6) \ \delta (\text{ppm}): 4.02 (s, 3\text{H}, \text{OCH}_3), 6.13 (d, 1\text{H}, \text{C5-H of pyridine}), 7.22-8.32 (m, 11\text{H}, \text{Ar-H}), 8.35 (s, 1\text{H}, \text{CH=NH}), 8.69 (d, 1\text{H}, \text{C4-H of pyridine}), 10.82 (s, 1\text{H}, \text{NH}), 11.81, (s, 1\text{H}, \text{NHCO}); \(^{13}\text{C}-\text{NMR} (75 \text{ MHz, DMSO-d}_6) \ \delta (\text{ppm}): 56.1, 104.6, 106.5, 118.3, 123.5, 124.1, 126.5, 127.5, 128.0 (3\text{C}), 128.6 (2\text{C}), 129.5, 130.8 (3\text{C}), 133.2, 142.5, 145.0, 153.4, 156.2, 161.5, 172.1; MS (EI, 70 eV) \( m/\text{z} \) (%) = 397 (M\(^+\), 9.30), 348 (100.00), 184 (20.00), 155 (22.70), 141 (24.00). Anal. Calcd for C\(_{24}\)H\(_{19}\)N\(_3\)O\(_3\) (397.43): C 72.53; H 4.82; N 10.57%. Found: C 72.49; H 4.80; N 10.58%. 

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Synthesis of 3-(2-methoxynaphthalen-1-yl)-5-oxo-4,5-dihydro-1H-pyrazole-4-carbonitrile (9). A mixture of compound 3 (0.53 g, 2 mmol) in DMF (10 mL) containing triethylamine (0.5 mL) was refluxed for 5 h, and then allowed to cool. The precipitate that formed was filtered off and recrystallized from EtOH to afford 9; orange powder; yield (76.3%); mp 260-262 °C; IR (KBr): ν/cm⁻¹ = 3190 (NH), 2217 (CN), 1671 (CO); ¹H-NMR (300 MHz, DMSO-d₆) δ (ppm): 3.65 (s, 1H, CH of pyrazole), 4.06 (s, 3H, OCH₃), 7.44-8.16 (m, 6H, Ar-H), 9.49 (s, 1H, NH); ¹³C-NMR (75 MHz, DMSO-d₆) δ (ppm): 39.4, 56.1, 108.6, 117.5, 118.2, 123.4, 124.5, 126.3, 128.3 (2C), 129.0, 133.6, 153.2, 155.1, 172.1; MS (EI, 70 eV) m/z (%) = 265 (M⁺, 17.40), 247 (26.10), 220 (39.10), 193 (47.80), 169 (91.30), 140 (100.00). Anal. Calcd for C₁₅H₁₁N₃O₂ (265.27): C 67.92; H 4.18; N 15.84 %. Found: C 67.90; H 4.19; N 15.81%.

Synthesis of 1-(2-cyanoacetyl)-5-hydroxy-3-(2-methoxynaphthalen-1-yl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (10). To a solution of compound 3 (0.53 g, 2 mmol) in DMF (10 mL), triethylamine (0.5 mL) and ethyl cyanoacetate (0.22 g, 2 mmol) were added. The reaction mixture was heated under reflux for 5 h, and then allowed to cool. The precipitate that formed was filtered off and recrystallized from EtOH to afford 10; white crystals; yield (74.7%); mp 210-212 °C; IR (KBr): ν/cm⁻¹ = 3333 (OH), 3190 (NH), 2264 (2CN), 1686 (C=O); ¹H-NMR (300 MHz, DMSO-d₆) δ (ppm): 3.97 (s, 3H, OCH₃), 4.23 (s, 2H, CH₂), 5.04 (s, 1H, C₅-H of pyrazole), 7.39-8.07 (m, 6H, Ar-H), 8.86 (s, 1H, NH), 10.50 (s, 1H, OH); ¹³C-NMR (75 MHz, DMSO-d₆) δ (ppm): 29.4, 55.1, 56.4 (2C), 115.2, 117.1 (2C), 118.2, 123.4, 124.5, 126.3, 128.2 (2C), 129.0, 133.6, 153.2, 169.5, 188.6; MS (EI, 70 eV) m/z (%) = 334 (M⁺, 15.60), 267 (28.60), 183 (100.00), 169 (39.00), 140 (36.40). Anal. Calcd for C₁₈H₁₄N₄O₃ (334.33): C 64.67; H 4.22; N 16.76%. Found: C 64.60; H 4.20; N 16.75%.

Synthesis of N-((2-methoxynaphthalen-1-yl)methylene)-3-oxo-3H-benzo[f]chromene-2-carbohydrazide (11). To a solution of compound 3 (0.53 g, 2 mmol) in DMF (10 mL), triethylamine (0.5 mL) and 2-hydroxy-1-naphthaldehyde (0.34 g, 2 mmol) were added. The reaction mixture was heated under reflux for 3 h, and then allowed to cool. The precipitate that formed was filtered off and recrystallized from EtOH to afford 11; yellowish brown powder; yield (84.7%); mp 178-180 °C; IR (KBr): ν/cm⁻¹ = 3245 (NH), 1713, 1680 (2C=O); ¹H-NMR (300 MHz, DMSO-d₆) δ (ppm): 4.05 (s, 3H, OCH₃), 7.26-8.17 (m, 12H, Ar-H), 8.46 (s, 1H, C₄-H of coumarin), 8.89 (s, 1H, CH=N), 11.79 (s, 1H, NH); ¹³C-NMR (75 MHz, DMSO-d₆) δ (ppm): 56.2, 106.2, 115.1, 117.0, 118.2, 122.1, 123.4 (2C), 124.0, 126.3 (2C), 128.4 (4C), 129.0 (2C), 130.2 (2C), 133.6, 143.2, 145.0, 150.5, 153.0, 159.4, 171.2; MS (EI, 70 eV) m/z (%) = 422 (M⁺, 1.20), 368 (7.30), 337 (90.30), 306 (37.50), 184 (37.70), 169 (100.00), 141 (48.40). Anal. Calcd for C₂₆H₁₈N₂O₄ (422.44): C 73.92; H 4.30; N 6.63%. Found: C 73.86; H 4.25; N 6.63%.

General procedure for the synthesis of 1-((2-methoxynaphthalen-1-yl)methyleneamino)-2-oxo-2,3-dihydro-1H-pyrrole-3-carbonitrile derivatives 12 and 13. A mixture of compound 3 (0.53 g, 2
mmol) and phenacyl bromide (0.39 g, 2 mmol) and/or chloroacetonitrile (0.15 g, 2 mmol) in DMF (10 mL) containing triethylamine (0.5 mL) was refluxed for 3 h. The formed solid product was filtered off and recrystallized from EtOH to give 12 and 13, respectively.

1-((2-Methoxynaphthalen-1-yl)methyleneamino)-2-oxo-5-phenyl-2,3-dihydro-1H-pyrrole-3-carbonitrile (12): yellow powder; yield (74.7%); mp 223-225 °C; IR (KBr): ν/cm⁻¹ = 2212 (CN), 1657 (C=O); ¹H-NMR (300 MHz, DMSO-d₆) δ (ppm): 4.06 (s 3H, OCH₃), 4.54 (d, J=2.5 Hz, 1H, C₃H of pyrrole ), 5.65 (d, J=2.5 Hz, 1H, C₄H of pyrrole ), 7.47-8.17 (m, 11H, Ar-H), 8.71 (s, 1H, CH=N); ¹³C-NMR (75 MHz, DMSO-d₆) δ (ppm): 38.2, 56.1, 97.0, 106.1, 115.5, 116, 118.1, 123.4, 124.5, 126.3, 128.3 (2C), 129.2 (2C), 130.3 (3C), 133.4, 136 (2C), 143.0, 153.2, 171.0; MS (EI, 70 eV) m/z (%) = 367 (M⁺, 12.90), 337 (88.10), 306 (48.60), 185 (12.90), 169 (100.00), 141 (55.00). Anal. Calcd for C₂₃H₁₇N₃O₂ (367.41): C 75.19; H 4.66; N 11.44%. Found: C 75.16; H 4.65; N 11.45%.

5-Amino-1-((2-methoxynaphthalen-1-yl)methyleneamino)-2-oxo-2,3-dihydro-1H-pyrrole-3-carbonitrile (13): yellowish brown powder; yield (78.7%); mp 252-254 °C; IR (KBr): ν/cm⁻¹ = 3439 (NH₂), 2212 (CN), 1657 (C=O); ¹H-NMR (300 MHz, DMSO-d₆) δ (ppm): 4.06 (s, 3H, OCH₃), 4.12 (d, J=2.5 Hz, 1H, C₄H of pyrrole ), 4.52 (d, J=2.5 Hz, 1H, C₃H of pyrrole ), 6.65 (s, 2H, NH₂), 7.36-8.16 (m, 6H, Ar-H), 8.71 (s, 1H, CH=N); ¹³C-NMR (75 MHz, DMSO-d₆) δ (ppm): 36.7, 56.1, 87.4, 108.0, 115.6, 118.2, 123.5, 124.1, 126.3, 128.3, 129.4, 133.4 (2C), 143.2, 145.6, 153.3, 169.4; MS (EI, 70 eV) m/z (%) = 306 (M⁺, 23.50), 285 (20.60), 206 (17.60), 183 (67.60), 169 (35.30), 154 (41.20), 140 (70.60). Anal. Calcd for C₁₇H₁₄N₄O₂ (306.32): C 66.66; H 4.61; N 18.29%. Found: C 66.63; H 4.58; N 18.26%.

Synthesis of 4-amino-N-((2-methoxynaphthalen-1-yl)methylene)-3-phenyl-2-thioxo-2,3-dihydrothiazole-5-carbohydrazide (14). To a stirred solution of 3 (5.3 g, 20 mmol), finely divided sulfur (0.65 g, 20 mmol) and triethylamine (2.5 mL) in DMF (20 mL), phenyl isothiocyanate (2.5 mL, 20 mmol) was added. The reaction mixture was heated under reflux for 6 h. The precipitate that formed was filtered off and recrystallized from EtOH to afford 14; Brown crystals; yield (68.3%); mp 242-244 °C; IR (KBr): ν/cm⁻¹ = 3423, 3345 (NH₂), 3245 (NH), 1657 (C=O), 1268 (C=S); ¹H-NMR (300 MHz, DMSO-d₆) δ (ppm): 4.06 (s, 3H, OCH₃), 6.69 (s, 2H, NH₂), 7.44-8.17 (m, 11H, Ar-H), 8.71 (s, 1H, CH=N); ¹³C-NMR (75 MHz, DMSO-d₆) δ (ppm): 56.1, 76.1, 106.3, 118.2, 123.4, 124.5, 126.8, 128.4 (2C), 129.5 (5C), 133.2 (3C), 143.1, 153.2, 159.1, 171.2, 184.6; MS (EI, 70 eV) m/z (%) = 434 (M⁺, 4.80), 409 (4.80), 368 (9.60), 337 (98.10), 306 (43.30), 169 (100.00), 141 (61.50). Anal. Calcd for C₂₂H₁₄N₄O₂S₂ (434.53): C 60.81; H 4.18; N 12.89%. Found: C 60.79; H 4.19; N 12.91%.

Synthesis of 2-cyano-3-((2-methoxynaphthalen-1-yl)methylene)hydrazinyl)-3-oxo-N-phenylpropanethioamide (16). To a stirred solution of powdered KOH (0.56 g, 10 mmol) in DMF (20
mL) compound 3 (2.67 g, 10 mmol) was added. After the mixture had been stirred for 0.5 h, phenyl isothiocyanate (1.2 mL, 10 mmol) was added and the stirring was continued at room temperature for 24 h. The reaction mixture was poured onto (100 mL) ice-cold water containing few drops of HCl (0.1 N). The solid product that separated was filtered off and recrystallized from EtOH to give compound 16; Orange crystals; yield (80.2 %); mp 184-186 °C; IR (KBr): ν/cm⁻¹ = 3440, 3187 (2NH), 2262 (CN), 1685 (C=O), 1270 (C=S); ¹H-NMR (300 MHz, DMSO-d₆) δ (ppm): 3.97 (s, 3H, OCH₃), 4.28 (s, 1H, CH), 7.44-8.16 (m, 11H, Ar-H), 8.71 (s, 1H, CH=N), 10.57 (s, 1H, NH), 11.45 (s, 1H, CONH); ¹³C-NMR (75 MHz, DMSO-d₆) δ (ppm): 56.2, 57.1, 106.5, 117.1, 118.6, 123.1, 124.4, 126.3 (3C), 128.4 (2C), 129.4 (3C), 133.2 (2C), 137.0, 142.7, 153.4, 171.2, 184.9; MS (EI, 70 eV) m/z (%) = 402 (M⁺, 14.30), 369 (57.10), 337 (100.00), 278 (44.60), 183 (91.30), 141 (40.20). Anal. Calcd for C₂₂H₁₈N₄O₂S (402.47): C 65.65; H 4.51; N 13.92%.

Synthesis of 2-cyano-⁴-(2-methoxynaphthalen-1-yl)methylene)-2-(5-oxo-3-phenylthiazolidin-2-ylidene)acetohydrazide (17). A mixture of compound 16 (4.02 g, 10 mmol) in DMF (20 mL), chloroacetyl chloride (1.13 g, 10 mmol) and few drops of triethylamine was refluxed for 5 h, and then allowed to cool. The formed solid product was collected by filtration and recrystallized from EtOH to afford compound 17; red crystals; yield (68.4 %); mp 245-247 °C; IR (KBr): ν/cm⁻¹ = 3189 (NH), 2263 (CN), 1686 (2C=O); ¹H-NMR (300 MHz, DMSO-d₆) δ (ppm): 3.99 (s, 3H, OCH₃), 4.22 ( s, 2H, CH₂), 7.16-8.41 (m, 11H, Ar-H), 8.71 (s, 1H, CH=N), 11.74 (s, 1H, NH); ¹³C-NMR (75 MHz, DMSO-d₆) δ (ppm): 56.1, 71.5, 88.6, 106.0, 117.0, 118.3 (3c), 120.8, 123.4, 124.5, 126.8, 128.0, 129.3 (3c), 133.1 (2c), 142.3, 145.0, 153.4, 162.2, 171.2, 192.4; MS (EI, 70 eV) m/z (%) = 442 (M⁺, 12.50), 369 (54.00), 337 (100.00), 278 (41.90), 185 (16.70). Anal. Calcd for C₂₄H₁₈N₄O₃S (442.49): C 65.65; H 4.10; N 12.66%. Found: C 65.10; H 4.08; N 12.62%.

General procedure for the synthesis of thiophene derivatives 18-20. To a solution of compound 16 (4.02 g, 10 mmol) in DMF (20 mL), the appropriate α-halo carbonyl compounds such as chloroacetone, phenacyl bromide and ethyl chloroacetate (10 mmol) and few drops of triethylamine were added. The reaction mixture was refluxed for 5 h, and then allowed to cool. The formed solid product was collected by filtration and recrystallized from EtOH to afford the corresponding thiophene derivatives 18-20, respectively.

5-Acetyl-4-amino-⁴-(2-methoxynaphthalen-1-yl)methylene)-2-(phenylamino)thiophene-3-carbohydrazide (18): yellowish brown powder; yield (87.7 %); mp 141-143 °C; IR (KBr): ν/cm⁻¹ = 3453, 3400 (NH₂), 3287 (2NH), 1690 (2C=O); ¹H-NMR (300 MHz, DMSO-d₆) δ (ppm): 2.50 ( s, 3H, CH₃), 3.98 (s, 3H, OCH₃), 5.95 (s, 2H, NH₂), 7.08-8.04 (m, 11H, Ar-H), 8.94 (s, 1H, CH=N), 10.53 (s, 1H, NH), 11.44 (s, 1H, CONH); ¹³C-NMR (75 MHz, DMSO-d₆) δ (ppm): 26.2, 56.1, 106.0 (2C), 118.5 (3C), 123.4 (2C), 124.5, 126.8 (2C), 128.3, 129.0 (3C), 131.2, 133.1, 139.0, 142.4, 146.0, 153.4, 169.0, 171.2, 186.1; MS (EI,
70 eV) \( m/z \) (%) = 458 (M\(^+\), 18.20), 441 (30.30), 301 (18.20), 243 (66.70), 217 (30.30), 184 (93.90), 169 (100.00). Anal. Calcd for \( \text{C}_25\text{H}_{22}\text{N}_4\text{O}_3\text{S} \) (458.54): C 65.49; H 4.84; N 12.22%. Found: C 65.42; H 4.84; N 12.20%.

4-Amino-5-benzoyl-N-((2-methoxynaphthalen-1-yl)methylene)-2-(phenylamino)thiophene-3-carbohydrazide (19): yellowish brown powder; yield (74.7%); mp 203-205 °C; IR (KBr): \( \nu/cm^{-1} = 3453, 3329 \) (NH\(_2\)), 3238 (2NH), 1690, 1660 (2C=O); \(^1\)H-NMR (300 MHz, DMSO-\( d_6 \)) \( \delta \) (ppm): 4.06 (s, 3H, OCH\(_3\)), 5.94 (s, 2H, NH\(_2\)), 7.47-8.17 (m, 16H, Ar-H), 8.94 (s, 1H, CH=N), 10.54 (s, 1H, NH), 11.44 (s, 1H, CONH); \(^13\)C-NMR (75 MHz, DMSO-\( d_6 \)) \( \delta \) (ppm): 56.2, 97.0, 106.2 (2C), 118.2 (3C), 120.0, 123.2, 124.4, 126.8, 128.8 (3C), 129.7 (5C), 132.1 (3C), 133.1, 138.5, 139.9, 142.6, 153.4, 161.0, 171.2, 178.1; MS (EI, 70 eV) \( m/z \) (%) = 520 (M\(^+\), 13.10), 347 (20.60), 319 (16.00), 269 (11.50), 183 (56.50), 169 (48.90), 140 (61.10). Anal. Calcd for \( \text{C}_{30}\text{H}_{24}\text{N}_4\text{O}_3\text{S} \) (520.61): C 69.21; H 4.65; N 10.76%. Found: C 69.16; H 4.61; N 10.70%.

Ethyl 3-amino-4-(2-((2-methoxynaphthalen-1-yl)methylene)hydrazinecarbonyl)-5-(phenylamino)thiophene-2-carboxylate (20): orange powder; yield (78.9%); mp 163-165 °C; IR (KBr): \( \nu/cm^{-1} = 3446, 3329 \) (NH\(_2\)), 3237 (2NH), 1730, 1660 (2C=O); \(^1\)H-NMR (300 MHz, DMSO-\( d_6 \)) \( \delta \) (ppm): 1.22 (t, J=7.2 Hz, 3H, CH\(_3\)), 4.00 (s, 3H, OCH\(_3\)), 4.37 (q, J=7.2 Hz, 2H, OCH\(_2\)), 5.89 (s, 2H, NH\(_2\)), 7.40-8.43 (m, 11H, Ar-H), 8.95 (s, 1H, CH=N), 10.45 (s, 1H, NH), 11.75 (s, 1H, CONH); \(^13\)C-NMR (75 MHz, DMSO-\( d_6 \)) \( \delta \) (ppm): 14.2, 56.2, 61.8, 106.5 (2C), 118.6 (3C), 120.0 (2C), 123.2, 124.1, 126.8, 128.0 (2C), 129.5 (3C), 133.1(2C), 139.5, 142.3, 153.5, 161.2, 169.3, 171.2; MS (EI, 70 eV) \( m/z \) (%) = 488 (M\(^+\), 15.20), 442 (16.90), 384 (16.00), 243 (18.10), 215 (47.40), 183 (100.00), 140 (37.10). Anal. Calcd for \( \text{C}_{26}\text{H}_{24}\text{N}_4\text{O}_4\text{S} \) (488.56): C 63.92; H 4.95; N 11.47%. Found: C 63.87; H 4.91; N 11.48%.

**ANTIMICROBIAL SCREENING**

**ANTIBACTERIAL ASSAY**

Antibacterial studies of newly synthesized compounds 3-20 were carried out against the representative panel of Gram-positive (\textit{Staphylococcus aureus} (MTCC – 96)) and Gram-negative (\textit{Escherichia coli} (MTCC – 443)). The activity of compounds was determined as per National Committee for Clinical Laboratory Standards (NCCLS) protocol using Müller-Hinton Broth. Primary screening was done first for antibacterial activity in six sets against \textit{E. coli} and \textit{S.aureus}, at different concentrations of 1000, 500, 250 \( \mu \)g/mL. The compounds found to be active in primary screening were similarly diluted to obtain 200, 125, 100, 62.5, 50, 25 and 12.5 \( \mu \)g/mL concentrations for secondary screening to test in a second set of dilution against all microorganisms. Inculum size for test strain was adjusted to \( 10^6 \) CFU/mL (Colony Forming Unit per milliliter) by comparing the turbidity (turbidimetric method). Müller-Hinton Broth was used as a nutrient medium to grow and dilute the compound suspension for test organisms. 2% DMSO was used as a diluent/vehicle to obtain the desired concentration of synthesized compounds and standard drugs to test.
upon standard microbial strains. Synthesized compounds were diluted to 1000 µg/mL concentration as stock solution. The control tube containing no antibiotic was immediately subcultured [before inoculation] by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of test organisms. The culture tubes were then incubated for 24 h at 36 °C and the growth was monitored visually and spectrophotometrically. 10 µg/mL suspensions were further inoculated on an appropriate media and growth was noted after 24 h and 48 h. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimal inhibitory concentration (MIC), i.e the amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Solvent had no influence on strain growth. The result of this was greatly affected by the size of inoculum. The test mixture should contain 10⁶ CFU/mL organisms. DMSO and sterilized distilled water were used as negative control while Ampicillin antibiotic (I U strength) was used as positive control. Standard drug used in the present study was "ampicillin" for evaluating antibacterial activity

ANTIFUNGAL ASSAY
The newly prepared compounds 3-20 were screened for their antifungal activity as primary screening in six sets against C. albicans at various concentration of 1000, 500, 250 µg/mL. The primary active compounds were similarly diluted to obtain 200, 125, 100, 62.5, 50, 25 and 12.5 µg/mL concentrations for secondary screening to test in a second set of dilution against fungi. The fungal activity of each compound was compared with Clotrimazole as a standard drug, which showed 5.8 µg/mL MIC against C. albicans. For fungal growth, in the present protocol, we have used Sabourauds dextrose broth at 28 °C in aerobic condition for 48 h. DMSO and sterilized distilled water were used as negative control while Clotrimazole (I U strength) was used as positive control.

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REFERENCES


