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A Convenient Route to the Synthesis of New Trisubstituted Pyrazolines and their Antimicrobial Activity

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ABSTRACT

In view of enormous applications associated with pyrazoline derivatives, a simple and accessible approach for the preparation of substituted pyrazolines is described. All synthesized new compounds were characterized by spectral and elemental analysis. Further the structures of the compounds (**6**) and (**7**) were obtained by single crystal x-ray diffraction studies. The products were screened *in vitro* for their antibacterial and antifungal activities against different organisms.

Keywords: Chalcones, dipolar, antifungal, antibacterial, MIC.

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INTRODUCTION

Five membered heterocycles such as pyrazolines are considered as useful synthons in organic synthesis for the construction of biologically potent molecules. Pyrazole moiety is a key motif in a wide number of natural and synthetic biologically active agents [1]. Literature reveals that pyrazolines were synthesised by various routes that includes by 1, 3-dipolar cycloaddition reaction of nitrile imines with alkenes [2-3], Vilsmeier-Hack reaction [4], and by one pot synthesis from α, β-unsaturated ketones [5-6], etc.

The synthesis of pyrazoline analogues has been a subject of consistent interest because of the wide range of applications in medicinal and pesticide chemistry. Pyrazolines have been reported to possess diverse biological applications such as anti-inflammatory [7], analgesic [8], antidepressant and anticonvulsant [9], anti-tumor [10], antioxidant and antimicrobial [11] activities. In view of broad spectrum of biological applications; and in continuation of our work on pyrazoles, we herein report a convenient route for the synthesis of pyrazolines and results of their *in vitro* antifungal and antibacterial activities.

MATERIALS AND METHODS

The reagents/chemicals used were obtained from Aldrich Chemicals, India and were used as such without further purification. Melting points were determined by open capillary method and are uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker supercon 400 MHz and Spect 500 MHz spectrophotometer using $CDCl_3$ as solvent and TMS as an internal standard. The Chemical shifts are expressed in δ ppm. Mass spectra were obtained on Shimadzu LCMS-2010A spectrophotometer. Elemental analysis was obtained on a Thermo Finnigan Flash EA 1112 CHN analyzer. Chromatographic separations were carried out on silica gel (70-230 mesh, Merck) column using hexane: ethyl acetate (9:1) as eluent.

The required intermediate chalcones (4-5) were prepared by the reaction of 2-acetyl thiophene (1) and aromatic aldehydes in alkali medium by a known procedure. Mixture of chalcones (4-5) (3 mmol), phenyl hydrazine hydrochlorides (3 mmol) and 3-4 pellets of KOH in ethanol (30 mL) was refluxed on a water bath for 3h. The progress of the reaction was monitored by TLC. After the completion of the reaction, the mixture was poured into ice cold water, the solid formed was filtered and washed thoroughly with ice cold water and dried. The solid obtained was recrystallized from ethanol to get target molecules (6-14) in good yield. The reaction pathway is illustrated in scheme-1.

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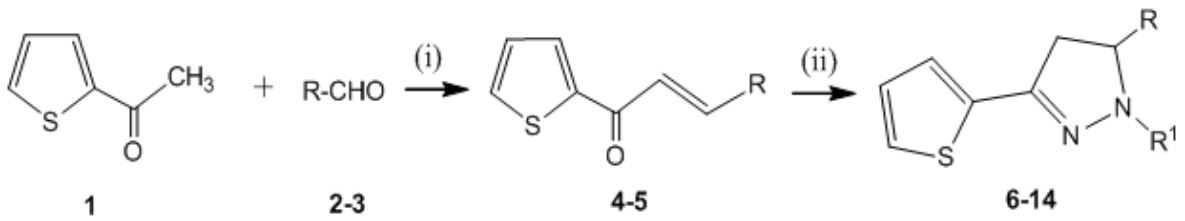

1 2-3 4-5 6-14

6. R = 4-$CF_3C_6H_5$, R^1 = $CONH_2$; 7. R = 4-$CF_3C_6H_5$, R^1 = $CSNH_2$;
8. R = 4-$CF_3C_6H_5$, R^1 = 4-ClC_6H_4; 9. R = 4-$CF_3C_6H_5$, R^1 = 2-ClC_6H_4;
10. R = 4-$CF_3C_6H_5$, R^1 = C_6H_5; 11. R = 3, 4-$(OCH_3)_2C_6H_3$, R^1 = $CONH_2$;
12. R = 3, 4-$(OCH_3)_2C_6H_3$, R^1 = 4-ClC_6H_4; 13. R = 3, 4-$(OCH_3)_2C_6H_3$, R^1 = 2-ClC_6H_4;
14. R = 3, 4-$(OCH_3)_2C_6H_3$, R^1 = 3-ClC_6H_4.

Reagents and Conditions: (i) $NaOH/C_2H_5OH$, RT, 2h.
(ii) $R^1NHNH_2.HCl/KOH/C_2H_5OH$, 70°C, 3h

Scheme-1: Synthetic pathway for the synthesis of trisubstituted pyrazolines

Minimum inhibitory concentrations (MIC's) of the test compounds (6-14) was done by broth dilution technique [12].

RESULTS AND DISCUSSION

Chemistry

***3-(Thiophen-2-yl)-5-(4-(trifluoromethyl)phenyl)-4, 5-dihydro-1H-pyrazole-1-carboxamide*, 6:**

Obtained from 1-(thiophen-2-yl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one, **4** (0.004 mmol) and semicarbazine hydrochloride (0.004 mmol) as white crystals in 88% yield. M.P. 90-93°C. ^{1}H NMR ($CDCl_3$): δ 3.09-3.14 (dd, 1H, C_4-H), 3.78-3.84 (dd, 1H, C_4-H), 5.01-5.04 (dd, 1H, C_5-H), 5.54 (s, 2H, -NH_2), 7.03-7.57 (m, 7H, Aromatic, thiophene ring). ^{13}C NMR ($CDCl_3$): δ 40.6 (1C), 67.1 (1C), 123.6 (1C), 124.2 (1C), 124.6 (2C), 125.3 (1C), 125.8 (2C), 127.2 (2C), 129.3 (1C), 146.0 (1C), 155.9 (1C), 159.7 (1C). MS (m/z): 340 (M+1) base peak. Anal. Calcd. for $C_{15}H_{12}F_3N_3OS$: C 53.09, H 3.56, N 12.38%; Found C 52.98, H 3.53, N 12.35%.

***3-(Thiophen-2-yl)-5-(4-(trifluoromethyl)phenyl)-4, 5-dihydro-1H-pyrazole-1-carbothioamide*, 7:**

Obtained from 1-(thiophen-2-yl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one, **4** (0.004 mmol) and thiosemicarbazine hydrochloride (0.004 mmol) as white crystals in 81% yield. M.P. 94-96°C. ^{1}H NMR ($CDCl_3$): δ 3.2 (d, 2H, C_3-H), 3.8 (t, 1H, C_2-H), 5.5 (s, 2H, -NH_2), 7-7.2 (dd, 2H, Ar-H), 7.36 (t, 1H, C_4-H thiophen ring), 7.37 (d, 1H, C_3-H thiophen ring), 7.4 (d, 1H, C_5-H thiophen ring), 7.5-7.6 (dd, 2H, Ar-H). MS (m/z): 356 (M+1) base peak. Anal. Calcd. for $C_{15}H_{12}F_3N_3S_2$: C 50.69, H 3.40, N 11.82%; Found C 50.71, H 3.39, N 11.79%.

***1-(4-Chlorophenyl)-3-(thiophen-2-yl)-5-(4-(trifluoromethyl)phenyl)-4, 5-dihydro-1H-pyrazole*, 8:**

Obtained from 1-(thiophen-2-yl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one, **4** (0.004 mmol) and phenylhydrazine hydrochloride (0.004 mmol) as white crystals in 74% yield. M.P. 112-114°C. ^{1}H NMR ($CDCl_3$): δ 3.05-3.08 (dd, 1H, C_4-H), 3.71-3.78 (dd, 1H, C_4-H), 5.07-5.08 (dd, 1H, C_5-H), 6.65-7.27 (m, 11H, Aromatic, thiophene ring). ^{13}C NMR ($CDCl_3$): δ 40.3 (1C), 60.4 (1C), 114.1 (2C), 124.2 (1C), 124.5 (1C), 124.8 (2C), 125.3 (2C), 125.8 (1C), 126.5 (1C), 127.1 (1C), 127.6 (1C), 129.2 (1C), 129.8 (2C), 142.3 (1C), 146.2 (1C), 154.6 (1C). MS (m/z): 409 (M+1, ^{37}Cl, 33), 407 (M+1, ^{35}Cl, 100 base peak). Anal. Calcd. for $C_{20}H_{14}ClF_3N_2S$: C 59.04, H 3.47, N 6.89%; Found C 58.98, H 3.43, N 6.88%.

***1-(2-Chlorophenyl)-3-(thiophen-2-yl)-5-(4-(trifluoromethyl)phenyl)-4, 5-dihydro-1H-pyrazole*, 9:**

Obtained from 1-(thiophen-2-yl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one, **4** (0.004 mmol) and 2-chlorophenylhydrazine hydrochloride (0.004 mmol) as white crystals in 72% yield. M.P. 96-98°C. ^{1}H NMR ($CDCl_3$): δ 3.09-3.12 (dd, 1H, C_4-H), 3.85-3.88 (dd, 1H, C_4-H), 5.12-5.16 (dd, 1H, C_5-H), 6.88-7.45 (m, 11H, Aromatic, thiophene ring). MS (m/z): 409 (M+1, ^{37}Cl, 33), 407 (M+1, ^{35}Cl, 100 base peak). Anal. Calcd. for $C_{20}H_{14}ClF_3N_2S$: C 59.04, H 3.47, N 6.89%; Found C 58.99, H 3.49, N 6.91%.

***1-Phenyl-3-(thiophen-2-yl)-5-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazole*, 10:**

Obtained from 1-(thiophen-2-yl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one, **4** (0.004 mmol) and phenylhydrazine hydrochloride (0.004 mmol) as white crystals in 82% yield. M.P. 103-105°C. ^{1}H NMR ($CDCl_3$): δ 3.09-3.12 (dd, 1H, C_4-H), 3.86-3.90 (dd, 1H, C_4-H), 5.28-5.33 (dd, 1H, C_5-H), 6.70-7.57 (m, 12H, Aromatic, thiophene ring). ^{13}C NMR ($CDCl_3$): δ 40.6 (1C), 60.2 (1C), 116.3 (2C), 120.5 (1C), 124.5 (1C), 124.8 (2C), 125.6 (2C), 125.9 (1C), 126.6 (1C), 127.3 (2C), 129.1 (1C), 129.6 (2C), 143.4 (1C), 146.0 (1C), 155.1 (1C). MS (m/z): 373 (M+1, base peak). Anal. Calcd. for $C_{20}H_{15}F_3N_2S$: C 64.50, H 4.06, N 7.52%; Found C 64.45, H 4.01, N 7.50%.

***5-(3, 4-Dimethoxyphenyl)-3-(thiophen-2-yl)-4, 5-dihydro-1H-pyrazole-1-carboxamide*, 11:**

Obtained from 3-(3, 4-dimethoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one, **5** (0.004 mmol) and semicarbazine hydrochloride (0.004 mmol) as white crystals in 77% yield. M.P. 88-90°C. ^{1}H NMR ($CDCl_3$): δ 3.22-3.25 (dd, 1H, C_4-H), 3.86 (s, 6H, OCH_3), 3.92-3.94 (dd, 1H, C_4-H), 5.02-5.05 (dd, 1H, C_5-H), 5.22 (s, 2H, NH_2), 6.84-7.48 (m, 6H, Aromatic, thiophene ring). ^{13}C NMR ($CDCl_3$): δ 40.2 (1C), 55.6 (2C). 66.2 (1C), 109.1 (1C), 118.4 (1C), 121.3 (1C), 124.4 (1C), 125.4 (1C), 127.1 (2C), 134.3 (1C), 147.2 (2C), 155.4 (1C), 159.9 (1C). MS (m/z): 332 (M+1, base peak). Anal. Calcd. for $C_{16}H_{17}N_3O_3S$: C 57.99, H 5.17, N 12.68%; Found C 57.95, H 5.11, N 12.69%.

***1-(4-Chlorophenyl)-5-(3,4-dimethoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole*, 12**:

Obtained from 3-(3, 4-dimethoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one, **5** (0.004 mmol) and 4-chlorophenylhydrazine hydrochloride (0.004 mmol) as white crystals in 81% yield. M.P. 122-124°C. ^{1}H NMR ($CDCl_3$): δ 3.09-3.12 (dd, 1H, C_4-H), 3.88 (s, 6H, OCH_3), 3.94-3.96 (dd, 1H, C_4-H),

5.18-5.20 (dd, 1H, C_5-H), 6.89-7.56 (m, 10H, Aromatic, thiophene ring). ^{13}C NMR ($CDCl_3$): δ 40.6 (1C), 55.2 (2C). 60.4 (1C), 108.2 (1C), 114.1 (2C), 118.2 (1C), 121.8 (1C), 124.2 (1C), 125.0 (1C), 126.4 (1C), 127.6 (2C), 129.0 (2C), 135.0 (1C), 142.6 (1C), 147.1 (1C), 148.9 (1C), 155.3 (1C). MS (m/z): 401 (M+1, ^{37}Cl, 33), 399 (M+1, ^{35}Cl, 100 base peak).Anal. Calcd. for $C_{21}H_{19}ClN_2O_2S$: C 63.23, H 4.80, N 7.02%; Found C C 63.18, H 4.83, N 7.00%.

***1-(2-Chlorophenyl)-5-(3, 4-dimethoxyphenyl)-3-(thiophen-2-yl)-4, 5-dihydro-1H-pyrazole*, 13**:

Obtained from 3-(3, 4-dimethoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one, **5** (0.004 mmol) and 2-chlorophenylhydrazine hydrochloride (0.004 mmol) as white crystals in 68% yield. M.P. 133-135°C. ^{1}H NMR ($CDCl_3$): δ 3.10-3.12 (dd, 1H, C_4-H), 3.83 (s, 6H, OCH_3), 3.89-3.91 (dd, 1H, C_4-H), 5.14-5.16 (dd, 1H, C_5-H), 6.84-7.52 (m, 10H, Aromatic, thiophene ring). ^{13}C NMR ($CDCl_3$): δ 40.1 (1C), 55.4 (2C). 60.3 (1C), 109.3 (1C), 114.5 (1C), 118.3 (1C), 121.1 (1C), 121.6 (1C), 123.8 (1C), 124.1 (1C), 125.6 (1C), 127.3 (2C), 127.8 (1C), 130.3 (1C), 136.3 (1C), 143.6 (1C), 147.2 (1C), 149.0 (1C), 155.1 (1C). Anal. Calcd. for $C_{21}H_{19}ClN_2O_2S$: C 63.23, H 4.80, N 7.02%; Found C C 63.19, H 4.76, N 6.98%.

***1-(3-Chlorophenyl)-5-(3, 4-dimethoxyphenyl)-3-(thiophen-2-yl)-4, 5-dihydro-1H-pyrazole*, 14:**

Obtained from 3-(3, 4-dimethoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one, **5** (0.004 mmol) and 3-chlorophenylhydrazine hydrochloride (0.004 mmol) as white crystals in 87% yield. M.P. 129-131°C. ^{1}H NMR ($CDCl_3$): δ 3.12-3.14 (dd, 1H, C_4-H), 3.85 (s, 6H, OCH_3), 3.90-3.92 (dd, 1H, C_4-H), 5.10-5.12 (dd, 1H, C_5-H), 6.70-7.46 (m, 10H, Aromatic, thiophene ring). Anal. Calcd. for $C_{21}H_{19}ClN_2O_2S$: C 63.23, H 4.80, N 7.02%; Found C C 63.20, H 4.74, N 6.99%.

The structure proofs of the synthesised compounds were obtained from spectral, elemental and x-ray diffraction studies. The structural assignments were made by NMR analysis by considering compound (6) as the representative compound. In its ^{1}H NMR spectra, H_a, H_b and H_c protons of the pyrazoline ring appeared as a doublet of doublet. The doublets of Ha appeared in the region δ 3.09-3.12 ppm; doublets of H_b appeared in the region δ 3.78-3.84 ppm; and that of H_c in the region δ 5.01-5.04 ppm. Doublets of H_a and H_b are due to diastereotopic nature of methylene protons. Among H_a, H_b and H_c protons, H_c is the most deshielded due to its close proximity to benzene ring. H_c couples not only with H_a but also with H_b and appears as doublet of doublet instead of a triplet. The methylene protons of pyrazoline ring (H_a and H_b) exhibited a typical ABX spin system with H_c as a doublet of doublets (Fig-1). All synthesised compounds showed similar splitting signals. Further all showed signals due to aromatic and substituent protons in the expected region.

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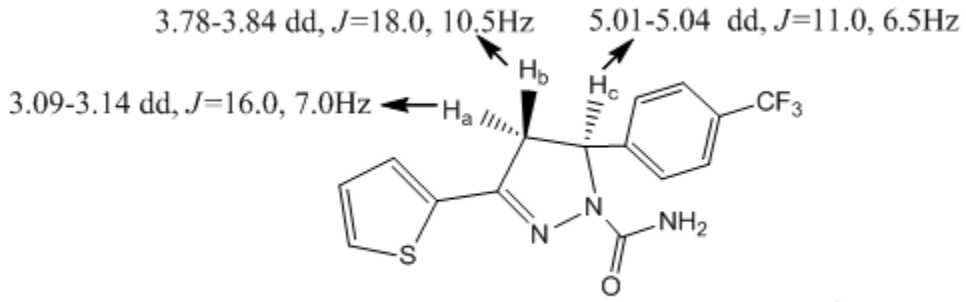

Fig-1: Proton chemical shifts and couplings of **6**

Further, the structures of (**6** and **7**) were confirmed by single crystal x-ray diffraction studies, which were depicted in ORTEP diagrams Fig-2 and Fig-3 respectively.

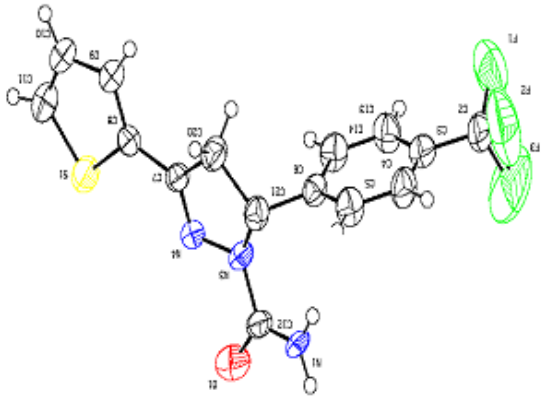

Fig-2: ORTEP diagram of **6** with 50% probability ellipsoids

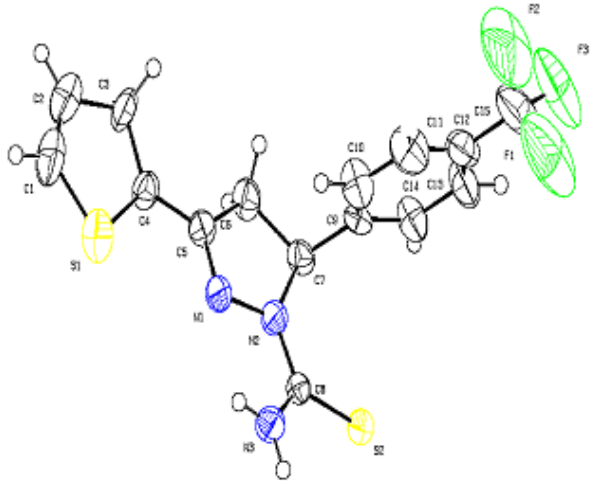

Fig-3: ORTEP diagram of **7** with 50% probability ellipsoids

The structural assignments were made by ^{13}C NMR analysis by considering representative compound (6). In its spectra, it showed a signal due to C_4-carbon at δ_c 40.6 ppm.,

for C_5-carbon at δ_c 67.1 ppm., and for C_3-carbon at δ_c 159.7 ppm of the newly formed pyrazoline ring. All the synthesised compounds (7-14) produced the signals due to C_3, C_4 and C_5 atoms in the same region. A collection of signals due to aromatic and substituent carbon atoms appeared in the expected region. The mass spectrum of all the synthesised compounds showed M+1 ion peak as base peaks, which confirmed the formation of the compounds. All the products (6-14) gave satisfactorily C, H, and N analysis, which further supported the structures of the synthesized products.

Antimicrobial activity

The results of minimum inhibitory concentration (MIC) of the synthesised compounds (**6-14**) against the fungi species *C. albicans*, *A. niger* and *A. flavus* are furnished in Table-1.

Table-1: MIC's of the synthesised compounds (6-14) against fungi species

Compound	Minimum inhibitory concentration (MIC's) in µg/mL*		
	C. albicans	*A. niger*	*A. flavus*
6	100	**	**
7	50	100	100
8	25	50	50
9	50	75	75
10	**	**	**
11	**	100	100
12	25	50	50
13	50	75	75
14	25	50	50
Amphotericin B	25	50	50

*Results are expressed as mean of three determinations (n=3);
**No activity observed even at a concentration of 200 µg/mL.

The investigated compounds showed different degrees of antifungal activity in relation to the tested microbial species. The extent of antifungal activity depended on the microorganism and the type of functional groups present in the molecule. The activity is considerably affected by the nature of the substitution on the newly formed pyrazoline ring. A close investigation of the *in vitro* antifungal activity profile of the substituted pyrazolines gives a clear picture of the SAR correlations among the compounds (6-14) under study. The compounds with halogen atoms on the aromatic ring exhibited promising antifungal activity against all the organisms tested and the rest of the series of compounds found moderately active. However, the compound (10) that possess no substitution on the aromatic ring showed its inability to exhibit antifungal activity against all the organisms even at a higher concentration of 200 µg/mL. Similarly, compound (6) was found inactive against *A. niger* and *A. flavus*, (11) was found inactive against *C. albicans.*

The minimum inhibitory concentration (MIC) results of the synthesised compounds (**6-14**) against the bacteria species *E. coli, S. typhimurium, B. subtilis* are summarized in Table-2.

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Table-2: MIC's of the synthesised compounds (6-14) against bacteria species

Compound	Minimum inhibitory concentration (MIC's) in µg/mL*		
	E. coli	*S. typhimurium*	*B. subtilis*
6	100	200	100
7	100	100	50
8	12	50	25
9	50	100	50
10	200	200	200
11	100	200	100
12	25	50	25
13	50	100	50
14	25	50	25
Ciprofloxacin	25	50	25

*Results are expressed as mean of three determinations (n=3);
**No activity observed even at a concentration of 200 µg/mL.

The test compounds (6-14) exhibited different degrees of antibacterial activity in relation to the tested microbial species. The activity is considerably affected by substituents present on the newly formed pyrazoline ring. The result of the study reveals that among the series of the compounds tested, the compounds (8, 12 and 14) bearing chloro substitution at meta and para positions of the aromatic ring exhibited remarkable antibacterial activity against the organisms tested. However, the remaining of the series of compounds synthesised showed good and/or relatively weak antibacterial activity.

CONCLUSION

In summary, a series of novel substituted pyrazolines have been synthesized in appreciable yields in an easy accessible method. Results of the antimicrobial activity reveal that, the synthesized compounds bearing chloro substitution on the aromatic ring act as potential antimicrobial agents. However, the structure-activity mode of action with the host cell remains of interest.

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