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Syntheses of 2,4,6-trisubstituted pyrimidine derivatives as a new class of antifilarial topoisomerase II inhibitors☆

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Abstract—A series of 21 compounds of trisubstituted pyrimidine derivatives have been synthesized and evaluated for their in vitro topoisomerase II inhibitory activity against filarial parasite *Setaria cervi*. Out of these, seven compounds (**8**, **11**–**14**, **25** and **28**) have shown 60–80% inhibition at 40 and 20 μg/mL concentration. Five compounds (**12**, **13**, **14**, **25** and **28**) exhibited 70–80% inhibition at 10 μg/mL concentration and three compounds (**13**, **14** and **28**) have shown 40–60% inhibition at 5 μg/mL concentration. All the above mentioned compounds have shown better topo II inhibitory activity than standard antifilarial drug (DEC) and enzyme topo II inhibitors (Novobiocin, Nalidixic acid).

1. Introduction

The lymphatic filariasis is a major vector born disease of the developing world and around 120 million persons are infected worldwide.[1] Within last 10 years, though significant progress has been made in the treatment and control strategies by introducing new diagnostic and monitoring tools, single annual or biannual dose therapy, combination therapy with diethylcarbamazine (DEC), ivermectin and albendazole,[1] lymphatic filariasis still continues to be a very severe problem due to lack of definite actions on adult worms. Among the various enzymes identified for drug development against parasitic diseases DNA topoisomerases have been chosen as a novel target for antifilarial drug development. DNA topoisomerases are the enzymes required for the replication, transcription and recombination of DNA. These enzymes play crucial roles in the organization of DNA within the cell nucleus as well as in its structure and function. The presence of ATP-dependent DNA topoisomerase II activity in the filarial parasites has been demonstrated in our laboratory.[2,3]

Previously in our laboratory some prototype molecules have been synthesized, which have shown adulticidal and antiinflammatory activities with additional DNA topoisomerase II inhibitory activity. These compounds possessed both macrofilaricidal and microfilaricidal actions combined with sterilizing effect due to DNA topoisomerase II inhibitory activity.[4,5] Earlier we have also reported some pyrido-indole and quinolone derivatives as novel antifilarial agents, some of them also exhibited topo II inhibitory activity.[6,7] In this manuscript we are reporting some novel trisubstituted pyrimidine derivatives as antifilarial topoisomerase II inhibitors.

2. Chemistry

Synthesis of compound **1** was carried out according to the literature procedure[8–10] with slight modifications. Syntheses of targeted compounds **8**–**28** is shown in Scheme 1.

Compounds **2**, **3** and **4** were synthesized by reacting compound **1** with guanidine hydrochloride and different imidines such as N-imidino morpholine hydrochloride and 4-amino benzimidine dihydrochloride, respectively,[10–13] in presence of NaH in dry DMF. Compounds **2**, **3** and **4** were oxidized to corresponding sulfones **5**, **6** and **7** in presence of *m*-chloroperoxy

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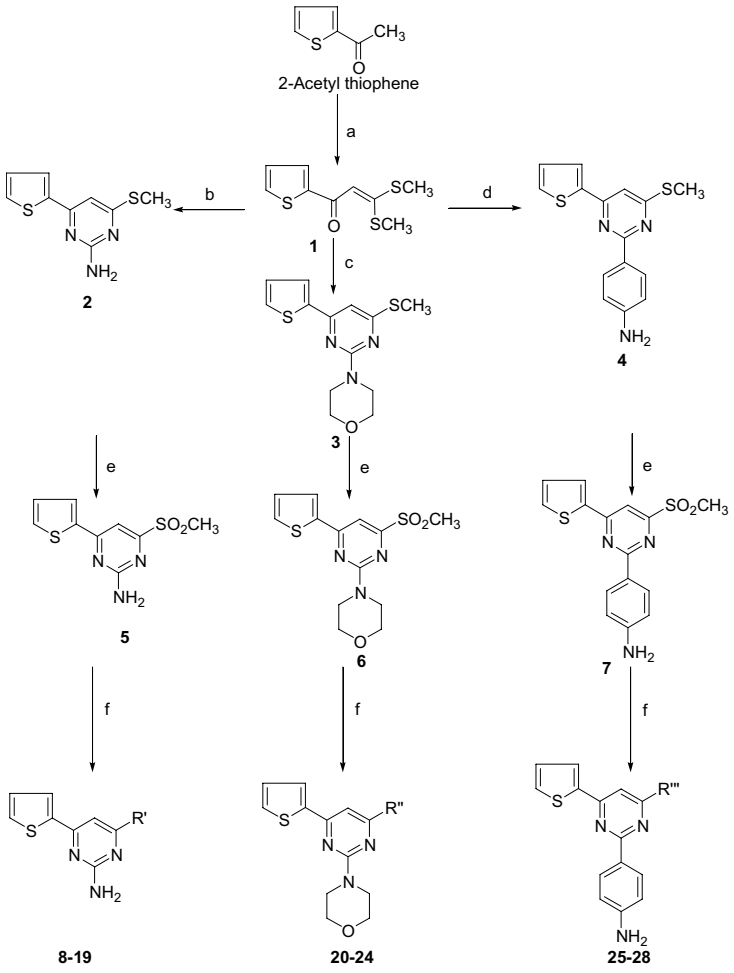


Scheme 1. Reagents and conditions: (a) CS_2, NaOH(s), MeI, THF, 0 °C–rt; (b) guanidine·HCl, NaH, DMF, 110 °C; (c) N-imidino morpholine·HCl, NaH, DMF, 110 °C; (d) 4-aminobenzimidine·2HCl, NaH, DMF, 110 °C; (e) *m*-CPBA, DCM, 0 °C–rt; (f) different amines, THF, 100 °C, in closed steel vessel.

benzoic acid (2.5 equiv). The sulfones **5**, **6** and **7** were subjected to nucleophilic substitution with various amines in closed steel vessel to yield targeted compounds **8**–**28**. All the synthesized compounds (Table 1) were well characterized by spectroscopic data such as mass, IR, NMR and elemental analysis.[15]

3. Biological activity

3.1. Material and methods

DNA topoisomerase was partially purified from the filarial parasites *Setaria cervi* according to slightly modified method of Pandaya et al.[14] Filarial parasites were homogenized in nuclei isolation buffer (NIB) (2.5 mM potassium phosphate buffer, pH 7.0; 2 mM $MgCl_2$; 0.1 mM EDTA; 1 mM EGTA; 1 mM DTT and 1 mM PMSF) and centrifuged at 3000*g* for 10 min at 4 °C. The pellet was washed with NIB and resuspended in NIB containing 4 mM EDTA; 0.35% (v/v) Triton x-100 and 0.375 M NaCl. The suspension was gently agitated for 15 min on ice and polyethylene glycol (9%, w/v) was added. The mixture was kept on ice for 1 h with occasional shaking and centrifuged at 10,000*g* for 30 min and 1,05,000*g* for 1 h.

Topoisomerase activity was estimated by monitoring the relaxation of supercoiled pBR322 DNA as reported previously (Pandya et al., 1999). Assay mixture (20 μL) contained 50 mM Tris–HCl, (pH 7.5); 50 mM KCl; 1 mM $MgCl_2$; 1 mM ATP; 0.1 mM EDTA; 0.5 mmM DTT; 30 μg/mL BSA; 0.25 μg pBR322 DNA and enzyme protein. The reaction mixture was incubated at 37 °C for 30 min and 5 μL stop buffer (0.25%, bromophenol blue, 1 M sucrose, 1 mM EDTA, 0.5% SDS) was added. The entire reaction mixture was loaded on 1% agarose gel and electrophoresed in 40 mM Tris–acetate buffer, pH 8.3, 1 mM EDTA at 20 V for 20 h. The gel was stained with ethidium bromide (0.5 μg/mL) and photographed in GDS 7500 UVP (Ultra Violet Products, UK) transilluminator. The effect of inhibitors on the enzyme activity was measured by incubating enzyme with inhibitor for 10 min at 37 °C and starting the reaction with addition of pBR322 DNA. The percent inhibition was measured by micro densitometry of the gel with GEL BASE/GEL BLOT PRO GEL analysis software program (Ultra Violet Products, UK).

Table 1.

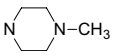
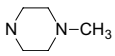
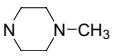
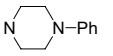
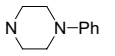
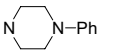
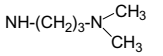
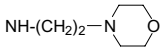
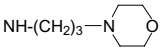
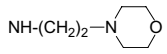
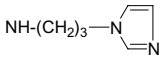
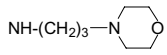
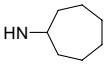
Compound no.	R′	Compound no.	R′	Compound no.	R″	Compound no.	R‴
8	NH–$(CH_2)_2$–CH_3	**14**		**20**		**25**	
9	NH–$(CH_2)_3$–CH_3	**15**		**21**		**26**	
10	NH–$(CH_2)_5$–CH_3	**16**		**22**		**27**	
11	NH–$(CH_2)_2$–OH	**17**		**23**		**28**	
12		**18**		**24**			—
13		**19**			—		—

Table 2. Topoisomerase II inhibitory activity against filarial parasite *Setaria cervi*

S. no.	Compounds	% Inhibition at different concentrations			
		40 μg/mL	20 μg/mL	10 μg/mL	5 μg/mL
1	**8**	60	60	NI	NI
2	**11**	60	60	NI	NI
3	**12**	80	80	80	NI
4	**13**	80	80	80	60
5	**14**	80	80	80	60
6	**25**	80	80	80	25
7	**28**	70	70	70	40
8	DEC (antifilarial)	45	10	NI	NI
9	Novobiocin (topo II inhibitor)	80	20	10	NI
10	Nalidixic acid (topo II inhibitor)	80	40	20	NI

NI = no inhibition.

4. Results and discussion

The effects of synthesized compounds are shown in Table 2. All the synthesized compounds were evaluated for their in vitro topoisomerase II inhibitory activity against filarial parasite *Setaria cervi*. Out of the 21 screened compounds, 7 compounds (**8**, **11**, **12**, **13**, **14**, **25** and **28**) exhibited 60–80% inhibition at 40 and 20 μg/mL concentration while the standard antifilarial drug (DEC) and topo II inhibitors (Novobiocin, Nalidixic acid) have shown 45–80% inhibition at a dose 40 μg/mL and 10–40% inhibition at a dose 20 μg/mL concentration. At 10 μg/mL concentration five compounds (**12**, **13**, **14**, **25** and **28**) exhibited 70–80% inhibition while the antifilarial drug (DEC) has shown no inhibition and topo II inhibitors, Novobiocin, Nalidixic acid, exhibited 10% and 20% inhibition, respectively. Four compounds (**13**, **14**, **25** and **28**) have shown 25–60% inhibition, where as standard drugs (DEC, Novobiocin and Nalidixic acid) showed no inhibition at 5 μg/mL concentration. Structure activity relationship of all the 21 screened compounds have given clear indication that amino group and 4-aminophenyl group at position-2 are very crucial in exerting topo II inhibitory activity against filarial parasite *Setaria cervi*. Most of the active compounds (**8**, **11**, **12**, **13** and **14**) either having amino group or 4-amino phenyl group (**25** and **28**) at position-2. The introduction of morpholino group at position-2 exerts no inhibitory effect on topo II enzyme of filarial parasite *Setaria cervi*.

5. Conclusion

In conclusion, we have synthesized and identified new trisubstituted pyrimidine derivatives as antifilarial topoisomerase II inhibitors. Some of these compounds exhibited significant topo II inhibitory activity as compared to standard antifilarial drug (DEC) and topo II inhibitors (Novobiocin, Nalidixic acid). Activity results indicates that compounds (**8 11**–**14**, **25** and **28**) can be utilized as lead molecules for further investigations and optimizing purpose for antifilarial chemotherapy.

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15. *General experimental procedure for the compound* **15**: The solution of compound **5** (1.27 g, 5 mmol) and 1-phenyl piperazine (0.81 g, 5 mmol) in dry THF was heated in closed steel vessel at 100 °C for 24 h. The solvent was removed under vacuum and resultant residue was dissolved in 100 mL $CHCl_3$. The organic phase was washed with H_2O (three times), dried over Na_2SO_4. The solution was concentrated and purified with column chromatography to afford compound **15** (1.20 g, 71.43%). By similar procedure described for compound **15**, compounds **8**–**28** were synthesized. *Compound* **15**: mp 139–141 °C; *m/z* (FAB-MS): 338 (M+1); IR (KBr): 3335, 3079, 2965, 2844, 1573, 1465, 1349 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 7.61 (d, 1H, $J = 3.55$ Hz, thiophene-H), 7.39 (d, 1H, $J = 4.84$ Hz, thiophene-H), 7.31 (d, 2H, $J = 7.89$ Hz, Ar–H), 7.09 (dd, 1H, $J = 4.82$, 3.52 Hz, thiophene–H), 6.98–6.86 (m, 3H, Ar–H), 6.35 (s, 1H, py–H), 4.77 (br s, 2H, NH_2), 3.81 (t, 4H, $J = 5.08$ Hz, NCH_2), 3.26 (t, 4H, $J = 5.10$ Hz, NCH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ 165.21, 164.25, 163.10, 149.34, 143.25, 129.64, 129.01, 128.17, 125.98, 120.68, 116.84, 89.10, 49.55, 44.35. Anal. Calcd for $C_{18}H_{19}N_5S$: C 64.07, H 5.68, N 20.75. Found: C 64.23, H 5.75, N 20.89. *Compound* **21**: mp 139–141 °C; *m/z* (FAB-MS): 408 (M+1); IR (KBr): 3087, 2935, 2865, 1600, 1575, 1465, 1379 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 7.61 (d, 1H, $J = 3.24$ Hz, thiophene–H), 7.38 (d, 1H, $J = 4.28$ Hz, thiophene–H), 7.31 (d, 2H, $J = 8.44$ Hz, Ar–H), 7.09 (dd, 1H, $J = 4.20$, 3.22 Hz, thiophene–H), 6.98–6.90 (m, 3H, Ar–H), 6.29 (s, 1H, pyr–H), 3.82–3.54 (m, 12H, OCH_2, NCH_2), 3.27 (t, 4H, $J = 5.10$ Hz, NCH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ 164.98, 164.15, 163.20, 148.56, 143.08, 129.69, 129.08, 128.32, 125.78, 120.15, 116.35, 89.21, 71.43, 59.18, 49.56, 44.24. Anal. Calcd for $C_{22}H_{25}N_5OS$: C 64.84, H 6.18, N 17.18. Found: C 64.52, H 6.34, N 17.27. *Compound* **26**: mp 120–123 °C (dec.); *m/z* (FABMS): 414 (M+1); IR (KBr): 3453, 3089, 2921, 2845, 1576, 1513, 1445, 1379 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 8.34 (d, 2H, $J = 8.52$ Hz, Ar–H), 7.74 (d, 1H, $J = 3.64$ Hz, thiophene–H), 7.43 (d, 1H, $J = 4.86$ Hz, thiophene–H), 7.29 (d, 2H, $J = 7.62$ Hz, Ar–H), 7.13 (dd, 1H, $J = 4.82$ and 3.62 Hz, thiophene–H), 7.01–6.87 (m, 3H, Ar–H), 6.69 (s, 1H, pyr–H), 6.74 (d, 2H, $J = 8.54$ Hz, Ar–H), 3.95 (t, 4H, $J = 5.10$ Hz, NCH_2), 3.87 (br s, 2H, NH_2), 3.34 (t, 4H, $J = 5.08$ Hz, NCH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ 164.10, 163.24, 158.70, 151.54, 149.07, 144.96, 130.24, 129.66, 129.14, 128.56, 128.22, 125.92, 120.66, 116.86, 114.85, 93.87, 49.56, 44.40. Anal. Calcd for $C_{24}H_{23}N_5S$: C 69.70, H 5.61, N 16.94. Found: C 69.35, H 5.76, N 16.83.